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Environmental effects on early life stages of American shad (Alosa sapidissima) and rainbow smelt (Osmerus mordax)

Kristen Fuda
University of New Hampshire, Durham

ENVIRONMENTAL EFFECTS ON EARLY LIFE STAGES OF AMERICAN SHAD (*ALOSA SAPIDISSIMA*) AND RAINBOW SMELT (*OSMERUS MORDAX*)

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KRISTEN FUDA

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BA, Roger Williams University, 2004

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Thesis Director, Dr. David L. Berlinsky,
Associate Professor of Zoology

Dr. W. Huntting Howell,
Professor of Zoology

Dr. James F. Haney,
Professor of Zoology

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ABSTRACT

ENVIRONMENTAL EFFECTS ON EARLY LIFE STAGES OF AMERICAN SHAD (*ALOSA SAPIDISSIMA*) AND RAINBOW SMELT (*OSMERUS MORDAX*)

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Recruitment of American shad (*Alosa sapidissima*) and rainbow smelt (*Osmerus mordax*) has steadily declined over the last few decades, possibly due to the construction of physical impediments to migration and increases in anthropogenic pollution. In order to elucidate environmental parameters influencing early life stages of anadromous fish, both laboratory and field studies were conducted. The effects of abiotic factors, including dissolved oxygen (DO), pH, salinity, nitrate, and phosphate, on hatch and survival of larval and juvenile American shad and rainbow smelt were examined in laboratory studies. Field studies on shad emigration were conducted in the Exeter River, and studies on smelt egg viability were conducted in the Winnicut and Squamscott Rivers.

Extremely low DO saturation (20%, 1.74 mg l-1) was found to be detrimental to shad larvae, and all levels at and below 80% (6.94 mg l-1) caused a reduction in egg viability. Low pH (4) reduced egg and larval viability. Larval survival decreased with increased salinity, but egg hatch was unaffected by salinities up to 30 ppt. Nitrates and phosphates had no effect on eggs or larvae.

Unlike shad, smelt were found to be more tolerant to various abiotic environmental conditions. Extreme DO levels (10%, 1.09 mg l-1), low pH (4), and high salinity (20-30 ppt) negatively affected smelt egg hatch, but nitrates and phosphates had no significant impact. Larval survival was reduced at pH levels at or below 5, but all other parameters (DO, salinity, nitrates, and phosphates) caused no detrimental effects on larval survival. In field studies, high siltation was observed in the Winnicut River and high fungal growth was observed in the Squamscott River.

The conditions simulated in the laboratory may result naturally from reduced flow or inadequate fishways that may reduce survival of emigrating young shad. The field studies on early life stages of rainbow smelt suggest siltation and fungal growth that may be intensified by flow alteration of dams, may be detrimental to hatch. These findings can be applied to restocking efforts in Great Bay, NH and other estuaries of the Northeast by focusing efforts on areas best suited for spawning.

INTRODUCTION

Anadromous fish hatch in freshwater streams and most travel into bays and estuaries as larvae or juveniles where they feed and grow until large enough to travel to the open ocean. Adults then live strictly in marine environments prior to the next spawning migration. This extensive migration leaves them vulnerable to human impacts in a variety of habitats. The most critical habitat, however, appears to be the freshwater spawning grounds, where poor water quality, low oxygen levels, industrial discharges, chemical pollution, dams and impoundments, and inadequate fishway facilities have been implicated in the reduction of anadromous stocks (Rulifson et al. 1981). In New Hampshire, two anadromous species that are particularly affected by these anthropogenic impacts are the American shad (*Alosa sapidissima*) and rainbow smelt (*Osmerus mordax*).

American shad

American shad belong to the herring family, Clupeidae. Their native range is from Newfoundland to Florida, but they are most abundant from southern New England to North Carolina (Ross 1991). Anglers seek shad for both the exciting fight during the catch and their delicious flesh and roe. Shad prey on copepods, small fish, fish eggs, and amphipods, while serving as prey to seals, sharks, bluefin tuna, kingfish, and porpoises (Scott and Crossman 1973).

While in riverine habitats, many larger fish feed on juvenile shad (Johnson and Dropkin 1992). In the open ocean, they are pelagic schoolers, and upon reaching sexual maturity, return to their natal rivers to spawn. They identify their natal rivers using rheotaxis, a behavioral orientation to water currents (Dodson and Leggett 1974). Males and females mature between 3-5 and 4-6 years of age, respectively (Leim 1924). Migration begins when river temperatures reach 12°C and continues until the waters exceed 20°C (Leggett and Whitney 1972). Throughout the duration of the spawning season, females can produce up to 600,000 semi buoyant eggs, which are oviposited at night in shallow, moving waters (Marcy 1972). The eggs are carried by the current until hatching, which occurs after 3 to 12 days depending on water temperature (Marcy 1976; Scott and Crossman 1973). In order to avoid saline water, shad must spawn far enough upstream so the eggs can drift and hatch before reaching tidally influenced or estuarine waters (Marcy 1976).

American shad were historically an important commercial fishery, but due to reduced stocks, commercial interest has substantially decreased. Populations in New England waters have been drastically reduced by damming, pollution, and exploitation, all of which have been occurring for over a hundred years (Ross 1991). Now, sport fisheries have become more significant in those rivers that still maintain active spawning populations (Weiss-Glanz et al. 1986). Summers and Rose (1987) found that shad stock abundance was strongly correlated to water quality. While the construction of dams has impeded the movement of anadromous fish for over a hundred years (NH Fish and Game 2002), greater

attention has been focused on upstream movement of adult fish rather than the downstream emigration of their progeny. Attaining a clear understanding of the factors that affect the survival of out-migrating juvenile American shad would significantly increase the effectiveness of restoration efforts.

While there have been many attempts to restore shad populations, the restoration of shad stocks in the Susquehanna River has undoubtedly been the most successful. St. Pierre (2003) described the history of the fishery in the Susquehanna, efforts taken for stock enhancement, and improvements in stocks seen recently. Nearly all of the shad spawning grounds in the Susquehanna River and its tributaries were eliminated following the construction of several dams in the early 1900s. In order to restore spawning populations in the river, both restocking and construction of fish passages were implemented. Restoration efforts initially focused on transferring eggs from the Susquehanna, Potomac, Connecticut, and Columbia Rivers, but were replaced by broodstock and larval supplementation in 1976. As the stocks improved, fish ladders were built at several dams to allow gravid adults to travel upriver. Since these efforts were made, stocks have increased from 1,546 in 1985 to 163,330 in 2000, and the number of juveniles has shifted from predominately hatchery-reared to wild-spawned. Even with this success, the stocking of larval shad will continue until the stocks can be maintained naturally. In order to assess the success of this project and make further improvements, many monitoring studies have been performed using radio transmitters and otolith markings on both adults and juveniles. These studies can further advance restoration by identifying spawning

aggregations, areas most appropriate for fishways, and evaluating the success of dam passage both upstream and downstream (St. Pierre 2003).

The restoration attempts underway in New Hampshire have not been as successful. Efforts have primarily centered on the Exeter River, because two fishways provide the greatest access to spawning grounds (NH Fish and Game 2002). Despite stocking gravid adults since 1980, populations have not increased and in 2001 had actually decreased from the previous years (NH Fish and Game 2002). Modifications to the Exeter fish ladder decreased the flow in 2001, enabling fewer adults to pass to the spawning grounds upstream of the dam (NH Fish and Game 2002). Restocking of gravid adults into the Exeter River ranged from 0 to 1,462 adult shad per year, and the number of adult returns ranged from 12 to163 (NH Fish and Game 2002). New Hampshire's restoration has not involved larval restocking, which was so successful in the Susquehanna restoration. More importantly, no studies have been performed to evaluate outmigration of adults or juveniles. Without further investigating emigration, the effort and resources of the current restoration in New Hampshire may not be successful.

Rainbow smelt

In addition to American shad, rainbow smelt are also of particular interest for restoration. Rainbow smelt, members of the family Osmeridae, are pursued for their fine flavor and use as a baitfish (Ross 1991; Frost and Trial 1993). Historically they ranged from Newfoundland to New Jersey, but are now only

found from southern Canada to Massachusetts (Ross 1991). Smelt are a pelagic, schooling fish found in cool, coastal, oceanic waters during the summer months. In the fall, smelt travel inshore to bays and estuaries to feed prior to their winter migration into freshwater tributaries. Larvae and juveniles feed on copepods, euphausiids, amphipods, polychaetes, and fish (Buckley 1989), while adults prey on small mummichogs, cunner, anchovies, sticklebacks, silversides, and alewives (Bigelow and Schroeder 1953). Both males and females become sexually mature at approximately 2 years of age, when they participate in nighttime spring spawning as the rivers thaw. A single female can produce 28,000-53,000 demersal eggs that attach to stable gravel-like substrate exposed to the current (Dudnik and Shchukina 1990). Eggs incubate for 10 to 21 days (Geffen 1990), then the hatched larvae drift downstream to deeper waters where they feed on zooplankton.

Rainbow smelt are one of the most important fish found in New Hampshire waters both as a recreational winter fishery and as a part of the riverine, estuarine, and oceanic ecosystems. Although numbers are not high enough to support the commercial fisheries that were sustained in Canada for over 100 years, the current populations are adequate to support a recreational fishery (Klein-MacPhee 2002).

The same dams affecting American shad are also restricting access to prime spawning grounds from migrating rainbow smelt. As smelt do not utilize fish ladders, they are forced to spawn in areas below the dams, possibly exposing progeny to harmful environmental conditions including: poor water

quality, tidal exposure, siltation, altered water flow, and overcrowding caused by reduced spawning grounds. Recent studies by Woodlot Alternatives Inc. (2004) evaluating the smelt spawning grounds below the Winnicut Dam suggested that without immediate habitat restoration smelt populations may be lost from this river. This river has experienced population declines since the beginning of the 20th century, but when the dam washed out prior to 1941, populations rebounded enough to support a recreational fishery (Woodlot Alternatives Inc. 2004). A new dam was constructed in 1957, but the fishway incorporated into this newer dam does not allow for the passage of smelt. Since the dam's construction, populations have continued to decline, possibly due to degradation of spawning habitat and poor water quality (Woodlot Alternatives Inc. 2004). It has been suggested that dam removal may facilitate restoration efforts.

Smelt populations in the Winnicut River are typical of the trends seen in populations throughout Great Bay, New Hampshire. Egg deposition declined from 1999 to 2003, and more egg surveys and evaluations on habitat, hatching success, and juvenile growth are needed (NH Fish and Game 2004). Understanding the influence environmental parameters have on hatch and survival of smelt will allow the utilization and maximization of ideal spawning grounds for restoration attempts.

Environmental influences on anadromous fish populations

Dissolved Oxygen

In the freshwater habitat, many environmental parameters including dissolved oxygen, pH, salinity, nitrates, and phosphates can affect early life stages of anadromous fish. Dissolved oxygen saturation is an environmental parameter that must be closely monitored in all aquatic systems, because the amount of available oxygen in water is much less than that in air.

Low levels of dissolved oxygen (hypoxia) negatively impact fish larvae in several ways. In areas of organic matter accumulation, the oxidation of the organic matter will exhaust the dissolved oxygen in the water (Stumm and Morgan 1996), reducing the amount of oxygen available to the fish. In addition, a reduction in water flow may result in poor water quality, including low dissolved oxygen levels in stagnant, above-dam impoundment areas (Langan and Jones 1996). At extreme low levels of dissolved oxygen, vendace (*Coregonus albula*) and whitefish (*Coregonus lavaretus*) embryos experienced bradycardia, a decrease in heart rate. This response occurred in vendace at a higher oxygen level than in whitefish, because vendace were shown to have a higher oxygen requirement than whitefish (Czerkies et al. 2002). In addition, hypoxic conditions caused a release of hatching enzymes, resulting in premature hatching. These hatching enzymes only dissolved one egg layer and some embryos were too weak to break through the remaining layer (Czerkies et al. 2001). Also, embryos of Atlantic salmon (*Salmo salar*) experienced reduced growth at low oxygen levels due to a decrease in absorption of yolk and other exogenous substances

(Hamor and Garside 1977). Larvae also experience negative reactions to hypoxic conditions. Artic char (*Salvelinus alpinus*) larvae that hatched in low dissolved oxygen water had higher heart rates and increased rhythmic ventilatory movements. Normally, salmonids at that age are thought to rely on cutaneous ventilation (McDonald and McMahon 1977; Rombough and Ure 1991).

Some fish are better adapted to live in areas of low oxygen. For example, hypoxia may cause acidosis (a depression in blood pH) as seen in rainbow trout (*Oncorhynchus mykiss*). Carp (*Cyprinus carpio*) appear to be better suited for low dissolved oxygen environments, however, as their blood pH increases and they develop GTP in their red blood cells to enable them to adapt to hypoxic conditions (Lykkeboe and Weber 1978; Sovio et al. 1980). Because depressed dissolved oxygen affects species differently, tolerance levels must be determined for each species to design more effective management practices.

pH

Dissolved oxygen is not the only parameter impacting fish; lowered pH in many aquatic systems has also become an increasing threat to many species. Emissions from industry have deposited chemicals (SO_2, NOx, and HCl) in both the gaseous and particulate form, which precipitate as acid rain (Mason 1989). Due to increased acidity, periodic episodes of high rainfall or snowmelt have been associated with acute mortalities of fish (Haines 1981). In addition to acid rain, nitrification from fertilizers and industrial effluent can further decrease the

pH of waterways (Hendrey 1987; Stumm and Morgan 1996). All these anthropogenic sources threaten to alter the pH of the waterways.

Low pH causes a variety of detrimental physiological defects. The net movement of sodium and chloride ions (Na^+ and Cl^- respectively) in and out of the fish is severely affected at a pH of 5, with reduced influx and increased efflux. The influx, especially of Na^+, is severely impacted and almost completely stopped by a pH of 4 (Wood 1989). The collapse in ion regulation is a major cause for the environmental toxicity of low pH, and this effect is delayed in hard water for freshwater species (Wood and McDonald 1982). Ionoregulatory failure further impacts blood chemistry by lowering ion levels in the extracellular fluid. This causes an osmotic gradient between blood plasma and red blood cells resulting in the net movement of ions out of the red blood cells and water into the cells. This swelling of red blood cells causes an increase in blood viscosity and blood pressure resulting in circulatory failure (Wood and McDonald 1982).

Low pH (4.2-4.7) may also lead to acidosis, which could prevent the increase in Na^+,K^+-ATPase activity fish need to osmoregulate in full-strength seawater (Saunders et al. 1983). Saunders et al. (1983) proposed that the inhibition of smoltification of Atlantic salmon may be due to these ionic imbalances increasing metabolic demand.

The Bohr (decrease in hemoglobin-oxygen affinity in low pH) and Root (decrease in hemoglobin saturation in low pH) effects caused hypoxia in brook trout (*Salvelinus fontinalis*) from the decrease in blood pH during acute exposure to low environmental pH (Packer and Dunson 1970; Packer 1979). Impaired

oxygen consumption varies with acid source, with a lesser effect in acid mine runoff, likely due to the additional metals in the water (Packer and Dunson 1972). In the early larval stages of Atlantic herring (*Clupea harengus*), low pH levels (6 and below), and likely acidosis, resulted in decreased muscle fiber volume, smaller mitochondria, and degenerated cristae, all of which would reduce mobility and lower viability of larvae (Bahgat et al. 1989). Acidosis, however, did not appear to be a direct cause of death in pH levels between 4 and 6 (Wood and McDonald 1982). Low pH may also cause a mucus buildup on the gills, further decreasing the ability for oxygen utilization (Packer and Dunson 1972). Despite its negative effects, low pH does not necessarily cause irreversible damage to the branchial mechanisms if sublethal levels are not reached (Holeton et al. 1983). Due to the variety of physiological abnormalities caused by low pH, a lethal level must be determined in order to better monitor the natural habitats.

Osmoregulation

Anadromous fish face a unique challenge osmotically as well. Because most are euryhaline, migrating through many environments with varying salinities, they must have adaptations to cope with both hypo and hyper-osmotic conditions. Freshwater fish need to uptake ions from the environment and retain them in their bodies. However, marine fish drink 0.2-1.5% of their body weight in order to prevent dehydration (Love 1980), leading to an increased need to excrete excess ions to maintain equilibrium.

One way fish osmoregulate is through chloride cells in the gill epithelia. When fish are in a freshwater environment, salt ions are taken in from the environment to replace those that are lost from their body. In order to take in these ions, excretion of acids (H^+ or NH_4^+) and bases (HCO_3^-), regulated by carbonic anhydrase, occur through the apical membrane of the chloride cell in exchange for Na^+ and Cl^- (Marshall 1995). The Na^+ and Cl^- ultimately enter the basal membrane of the chloride cell by Na^+, K^+ ATPase or chloride channels, respectively (Marshall 1995). Fish in a saltwater environment must rid the body of excess Cl^-. In the chloride cells, the Na^+, K^+ ATPase creates an electrochemical gradient, driving the transport of Na^+ and Cl^- across the basal membrane. The accumulation of Cl^- in the chloride cell leads to the loss of Cl^- through ion channels in the apical membrane (Marshall 1995).

Salinity tolerance in anadromous fishes is associated with the development from the early life to the adult stage when fish are migrating from freshwater to increased salinities. During metamorphosis, American shad were shown to have a 2.8-fold increase in gill index and a 10-fold increase in gill Na^+,K^+-ATPase activity (Zydlewski and McCormick 1997a). The increase in Na^+,K^+-ATPase activity with seawater acclimation is similar to that of other euryhaline species (Zydlewski and McCormick 1997a; Ewing et al 2001). The increase in Na^+,K^+-ATPase may also be correlated with water temperatures encountered during emigration (Zydlewski and McCormick 1997b). In addition to an increase in gill formation and Na^+,K^+-ATPase activity, an increase in chloride cell density during the larval-juvenile transition may be necessary for

osmoregulation adaptation for hyperosmotic conditions (Zydlewski and McCormick 2001). Atlantic salmon smolts also had chloride cells of increased volume preparing them physiologically to enter saltwater conditions (Pisam et al. 1988). Zydlewski and McCormick (2001) suggested this increase in chloride cell number and size is energetically expensive to late migrants and directly interferes with respiration and other gill functions, leading to reduced performance and increased mortality.

A second way fish can regulate ion transport is through renal excretion. Reabsorption of ions occurs in the nephrons of fish in both fresh and saltwater; however, there is less absorption of ions in saltwater fish due to a higher osmotic gradient. This allows a greater amount of ions to be excreted in saltwater fish (Marshall 1995). As rainbow trout prepare for the change in salinity, they experience a decline in excretion, both of water and electrolytes, likely due to a reduced glomerular filtration rate. The tubular absorption and secretion, however, was not found to change (Holmes and Stainer 1966).

Anadromous fish eggs face less osmotic pressure than larvae and juveniles, because they are typically found in freshwater. If adults are forced to spawn close to the mouth of a river, however, eggs may be exposed to increased salinities. Because eggs have yet to develop the functional osmoregulatory organs, they may rely on processes at the cellular level as opposed to the organ level (Shen and Leatherland 1978). In early stages of egg development, the chorion and perivitelline fluid appear to be responsible for ion exchange (Rudy and Potts 1969). At the eyed stage, however, sodium permeability occurs in the

vitelline membrane making the embryo vulnerable to osmotic pressures (Rudy and Potts 1969). At the later stages of development, the embryo may be able to secrete Na+ into the perivitelline fluid to osmoregulate (Shen and Leatherland 1978). Increasing salinity and the associated need for greater osmoregulation is one of the greatest challenges facing emigration of anadromous fish during their early life stages.

Nitrates

Nitrates can be found in rivers from natural sources through atmospheric deposition, but increasing anthropogenic sources have led to increasing concern. Nitrates can enter the waterways through municipal and industrial waste; septic tanks; water discharges from mining; agricultural, feedlot, and urban runoff; fertilizers; landfill leachate; storm sewer overflow; and vehicular exhaust (National Research Council 1972; Environment Canada 2003). Nitrates have been implicated as an endocrine disrupter, which explains the indirect effect nitrates were suggested to have on egg size through adult female ovaries (Yannopoulos 1978; Guillette and Edwards 2005). However, nitrates can have direct impacts on early life stages of fish. Juvenile medaka (*Oryzias latipes*) exposed to 100 and 125 mg l-1 nitrate were shown to have appetite loss; lethargic behavior; toxic effects in gills, intestinal ampulla, liver, and kidney; cellular disorders; metabolic disturbances; and curvature of the spinal column (Shimura et al. 2004). Hypoxia also increases the toxicity of nitrogen compounds

(NO_3, NO_2, and NH_3) because of ion conversion, and greater accumulation (Tilak et al. 2002).

Phosphates

Phosphates have also been shown to affect early life stages of fish. They enter the water through runoff from agriculture and pesticides as well as sewage treatment facilities (Manahan 2000). Effects of phosphates on early life stages of fish have not been extensively studied, but phosphates have been shown to cause deformities in cell differentiation and morphogenesis resulting in decreased locomotion and survival (Toor et al. 1983). In addition to the direct effects on fish, phosphates also cause excessive algal growth, which leads to the depletion of dissolved oxygen in the water (Manahan 2000).

In rivers feeding Great Bay, levels of dissolved oxygen, pH, salinity, nitrates (as N), and phosphates (orthophosphate as P) can reach 3.36 mg l-1 (38.7%), 4.78, 10.9 ppt, 0.317 mg l-1, and 0.7203 mg l-1 respectively (NHDES National Coastal Assessment Tidal Water Quality Monitoring Data and Ambient River Monitoring Program). Elucidating the impacts of these environmental parameters (dissolved oxygen, pH, salinity, nitrates, and phosphates) on early life stages of American shad and rainbow smelt can enhance restoration attempts by focusing on areas that are conducive to hatch and survival. As a result, the long-term costs of restoration may be reduced while providing immediate recreational fishing and business-related opportunities.

CHAPTER I

AMERICAN SHAD (*ALOSA SAPIDISSIMA*)

Abstract

Restoration efforts for anadromous fish species have mainly focused on upstream movement of adult fish rather than downstream emigration of their progeny. Low flow conditions, resulting from water withdrawals and decreased discharge, can lower dissolved oxygen (DO) levels, decrease water quality, and impede out-migration of stocked juvenile fish. The effects of environmental conditions, including DO, pH, salinity, nitrate, and phosphate levels, on hatch and survival of larval (2 days post hatch) and juvenile (21 and 120 days post hatch) American shad (*Alosa sapidissima*) were examined in laboratory experiments. Extremely low dissolved oxygen concentrations (20%, 1.74 mg l-1) were found to be detrimental to larvae, while all levels at and below 80% (6.94 mg l-1) caused a reduction of viability in eggs. A pH of 4 reduced viability of eggs and survival of larvae. Larval survival decreased with an increase in salinity, but eggs were unaffected by salinities up to 30 ppt. Nitrates and phosphates showed no effect on eggs or larvae. To examine impacts of dams on emigration of juveniles, approximately 234,000 oxytetracycline-marked larval shad were released at two sites along the Exeter River, NH. Biweekly seining was performed to recapture released individuals. In addition, DO in the Exeter Dam impoundment was

monitored during emigration using a YSI datasonde. Field studies suggest decreased survival of stocked emigrants passing over the Pickpocket Dam, and YSI data revealed depressed DO levels in the Exeter Dam impoundment. These results can aid in more efficiently directing restoration efforts.

Introduction

Historically, American shad (*Alosa sapidissima*) supported an important commercial fishery, but commercial interest waned with population declines. Today recreational fishing is more significant in those rivers that still maintain active spawning populations (Weiss-Glanz et al. 1986). Shad are also an important part of riverine, estuarine, and oceanic ecosystems, as they prey on copepods, small fish, fish eggs, and amphipods, while serving as prey to seals, sharks, bluefin tuna, kingfish, and porpoises (Scott and Crossman 1973).

At sexual maturity, adults return to natal rivers to spawn. Migration begins when the river temperatures reach 12°C and continues until the temperature exceeds 20°C (Leggett and Whitney 1972). Females produce up to 600,000 semi buoyant eggs that are carried by the current until hatch, which occurs in 3 to 12 days depending on water temperature (Marcy 1976, Scott and Crossman 1973). Out-migration of juvenile American shad generally occurs eighty days post hatch (O'Donnell 2000).

The anthropogenic influences on the rivers in which shad spawn may be contributing to a decline in stocks by negatively impacting recruitment. The dams along these rivers can be lethal by blocking upstream migration of adults (Beasley and Hightower 2000). In rivers where fish ladders allow adult upstream migration, including the Exeter River (Exeter, New Hampshire) the dams may impede emigration of progeny. Kynard and Buerkett (1997) suggested migration of early life stages is normally not a problem, because they are carried by

sufficient water flow. The Exeter River, however, experiences periods of low flow, which coincide with juvenile shad emigration. This decrease in flow is caused by the combination of low seasonal flow and water withdrawals for anthropogenic purposes. The result may not only be a lack of dam overflow, but also poor water quality, including low dissolved oxygen levels in stagnant, above-dam impoundment areas (Langan and Jones 1996). Therefore, no matter how successful restoration efforts are at fostering reproduction above a dam (i.e.- stocking gravid adults, eyed eggs, or hatchery reared fry), significant juvenile mortality can occur as a result of poor water quality in the impoundment area. The restoration of anadromous fish on the Connecticut River was significantly delayed by the lack of effective downstream passage (Gephard and McMenemy 2004).

The Atlantic States Marine Fisheries Commission indicated that man-made perturbations in river flow are responsible for the drastic declines of alosine stocks (ASMFC 1999). The Fishery Management Plan for American shad and river herring reports a dissolved oxygen standard of not less than 5 mg l-1 at any time during this summer nursery phase for juveniles (ASMFC 1985). Dissolved oxygen levels in the impoundment in downtown Exeter, NH, have been documented to fall below the accepted standard for weeks at a time during the fall emigration period of American shad (Langan and Jones 1996). During these periods, juvenile alosids that would normally emigrate may become trapped in sub-optimal conditions that prevent them from completing their emigration. In laboratory studies, American shad eggs and larvae were also shown to be

extremely sensitive to fluxes in environmental conditions such as pH and temperature (Leach and Houde 1999). The purpose of this research was to investigate the impacts of environmental parameters on hatch and survival of American shad eggs and larvae. Understanding the parameters that negatively effect early life stages of shad will allow managers to properly allocate money, time, and resources to maximize restoration efforts.

Materials and Methods

Environmental impact on egg hatch and early larval survival

Shad eggs were obtained from tank spawning fish held at the Waldoboro Shad Hatchery in Waldoboro, Maine. The adult broodstock were captured from the Lawrence, Massachusetts fishlift along the Merrimack River in 2005 and held in captivity for less than four weeks prior to spawning. Newly spawned eggs were held in MacDonald jars with supplemental air until transport to the Aquaculture Research Center at the University of New Hampshire, Durham, New Hampshire in July 2005.

Hatch and larval survival were tested under varying dissolved oxygen, pH, salinity, nitrate, and phosphate levels in triplicate. To examine hatch, twenty eyed eggs (1 day post fertilization) were incubated in 1 l glass beakers with 800 ml of non-chlorinated well water and supplemental aeration (except dissolved oxygen treatments) at 21 ± 1°C until hatch (3-5 dpf). Viability of eggs was quantified just prior to hatch by visually examining the eggs. Viable eggs are transparent with moving embryos whereas nonviable eggs are opaque. Eggs not used in hatch experiments were incubated in MacDonald jars at 21 ± 1°C with supplemental aeration until hatch for larval experiments. Twenty, two days post hatch (dph) larvae were placed in 2 l blue plastic beakers for three days in 1.5 l of 5 ppt non-chlorinated water at 21 ± 1°C to assess early larval survival. Dissolved oxygen levels of 10% (0.9 mg l-1), 20% (1.7 mg l-1), 40% (3.5 mg l-1), 60% (5.2 mg l-1),

80% (6.9 mg l-1), and 100% (8.7 mg l-1, control) for hatch studies were obtained by bubbling gaseous nitrogen into the beakers. Larvae were reared in water with 20% (1.7 mg l-1), 40% (3.5 mg l-1), 60% (5.2 mg l-1), and 100% (8.7 mg l-1, control) dissolved oxygen saturation. Dissolved oxygen was monitored with a Handy Gamma meter (OxyGuard International, Birkerød, Danmark) and adjusted twice daily by adding gaseous nitrogen. To test the effects of low pH, sulfuric acid was added dropwise into beakers until a pH of 4, 5, 6, and 7 (control) was reached and validated with a pH meter PHAST-CHEK Pocket (VWR, West Chester, PA) for both hatch and larval studies. pH was monitored and adjusted daily by the addition of sulfuric acid. Salinities of 0 (control), 5, 10, 15, 20, and 30 ppt were attained by the addition of Instant Ocean™ and validated with a refractometer (Spartan Refractometers, Tokyo, Japan) for both hatch and larval studies. For hatch experiments, sodium nitrate was added to obtain nitrate concentrations of 0 (control), 3.65, 14.59, 29.18, and 58.35 mg l-1, and for larval studies concentrations of 0, 29.18, and 58.35 mg l-1 were tested. Phosphates were tested at 0 (0 mg l-1 phosphate, control), 0.1 (0.04 mg l-1 phosphate), 5 (2.08 mg l-1 phosphate), 10 (4.17 mg l-1 phosphate), and 20 mg l-1 (8.33 mg l-1 phosphate) potassium phosphate dibasic trihydrate for hatch studies, and only 0, 10, and 20 mg l-1 for larval studies.

Dissolved oxygen impacts on juvenile growth and survival

In July 2004, larval shad were obtained from the Waldoboro Shad Hatchery in Waldoboro, Maine, where eggs were held in MacDonald jars with

aeration until hatch, and transported to the Aquaculture Research Center (ARC) at the University of New Hampshire. Eggs were incubated as described above, and larvae were held in three 780 l blue plastic tanks with oxygenated well water (2 ppt NaCl, 150 ppm $CaCl_2$, 18°C). Larvae were initially fed *Artemia* and a combination of *Artemia* and Lansy NRD 2/4 (INVE AMERICAS, Inc., Salt Lake City, Utah) by 25 dph. Tanks were siphoned and water changed every three days.

To determine the effect of low oxygen levels on survival, ten juvenile American shad (21 dph) were housed in 8-liter blue plastic, cylindrical tanks containing 5 liters of oxygenated well water (2 ppt NaCl, 150 ppm $CaCl_2$, 24°C) and acclimated for 24 hours. During the acclimation period, fish were fed *Artemia* and received continuous aeration. Following acclimation, supplemental aeration was stopped and dissolved oxygen was displaced slowly by continuously (chronic) bubbling gaseous nitrogen into the water for 120 min until 60% (5.0 mg l-1) saturation was obtained and 315 min until oxygen levels of 20% (1.7 mg l-1) and 40% (3.3 mg l-1) saturation were obtained. Fish reared with supplemental aeration (100% saturation=8.3 mg l-1) served as controls, and each treatment was tested in triplicate. Mortality was recorded when the desired oxygen levels were first obtained, and after 3 and 24 hours. This study was repeated with fish of the same age, but a more abrupt (acute) addition of nitrogen to all treatment groups was added over 90 min to obtain oxygen levels of 20% and 40% saturation, while 100% served as the control. Similar trials were also conducted with 120 dph shad at 18°C in 12% (1.1 mg l-1), 18% (1.7 mg l-1), and 100% (9.2

mg l-1, control) dissolved oxygen saturations.

To test the effect of low oxygen levels on growth, 28 dph juvenile shad were reared as above for 12 days with 40% (3.3 mg l-1), 60% (5.0 mg l-1), and 100% (8.3 mg l-1, control) oxygen saturation. Each tank housed 35 fish, and each treatment was tested in triplicate. Water changes were performed at two-day intervals and water quality (ammonia, nitrite) was tested every third day. Dissolved oxygen was monitored with a Handy Gamma meter (OxyGuard International, Birkerød, Danmark) and adjusted twice daily. Fish were fed a combination of *Artemia nauplaii* and Lansy. A minimum of six fish from each tank were removed and measured on days 4, 8, and 12 of the study using ImageJ (U.S. National Institutes of Heatlh, Bethesda, Maryland) analysis of pictures taken by a PowerShot S40 using Remote Capture (Canon USA Inc., Lake Success, NY) mounted to a Leica S8APO (Leica Microsystems Inc., Bannockburn, IL).

Data analysis

Data were analyzed by ANOVA ($p<0.05$) using JMP Software (SAS Institute Inc., Cary, NC). All percent data was arcsine square root transformed. A Tukey-Kramer post hoc test was performed to determine significant differences among treatments.

Impact of dams on emigration of shad juveniles

The impact of dams and their impoundments on juvenile American shad

emigration was examined by obtaining, releasing, and re-capturing marked juvenile American shad in two distinct reaches of the Exeter River (Figure I.1). Feeding American shad juveniles, batch marked with oxytetracycline, either once or twice to distinguish fish from each release site were obtained from the Waldoboro shad hatchery in Waldoboro, ME in July 2004. These fish were transported to the Exeter River in 757 l tanks. Approximately 100,000 double marked juveniles were released above the first dam (Exeter) and 134,000 single marked juveniles were released above the second dam (Pickpocket) (Figure I.1).

Capture of American shad juveniles was conducted using a 18.3 m bag seine with 6.4 mm stretch mesh. Bi-weekly seining operations from 13 August to 25 October 2004, were conducted from a boat using semi-circular hauls deployed into the current. The areas sampled (Figure I.2) were determined using maps and prior experience of NH Fish and Game biologists gained during a similar study conducted in 1991 (NH Fish and Game 1992).

All recaptured American shad juveniles were enumerated, measured for length to the nearest mm using a ruler (Connecticut Valley Biological Supply Co., Southampton, MA), and stored at -20°C. After field operations were completed, juvenile shad were thawed for otolith extraction. Otoliths (sagittae) were extracted using a dissecting microscope, probe, and forceps. Each of the two otoliths was placed in a small drop of deionized water on a microscope slide to rinse off any remaining tissue. The otoliths were then mounted on a microscope slide using KrazyGlue® superglue (Elmer's Products Inc., Columbus, Ohio). Each slide was examined on the Zeiss Axioplan II Epi-Fluorescent Microscope

mounted with a high-resolution camera (Zeiss AxioCam MR) and then analyzed using the AxioVision 4.3 software package (Zeiss) for oxytetracycline markings.

Ambient environmental conditions in the Exeter River impoundment were assessed using a YSI 6000 data-logger (YSI inc., Yellow Springs, Ohio). The data-logger was deployed for one-week intervals and programmed to collect data every fifteen minutes from 17 August 2004 through the end of October 2004. The instrument was deployed from the Exeter fish ladder at a depth of one meter.

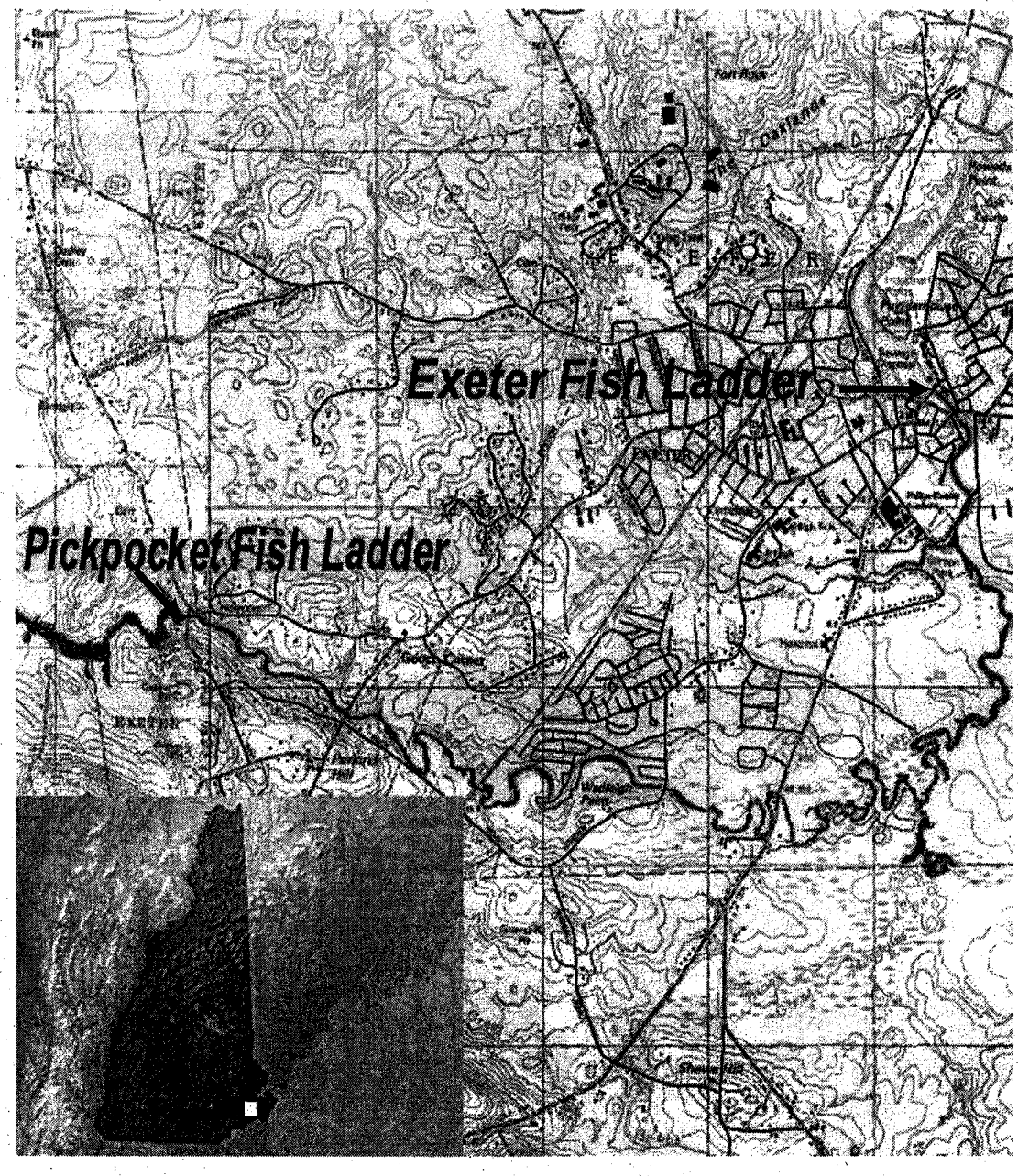

Figure I.1. Map of study area of Exeter River, New Hampshire. The river mouth in the upper right portion of the map leads to Great Bay, NH.

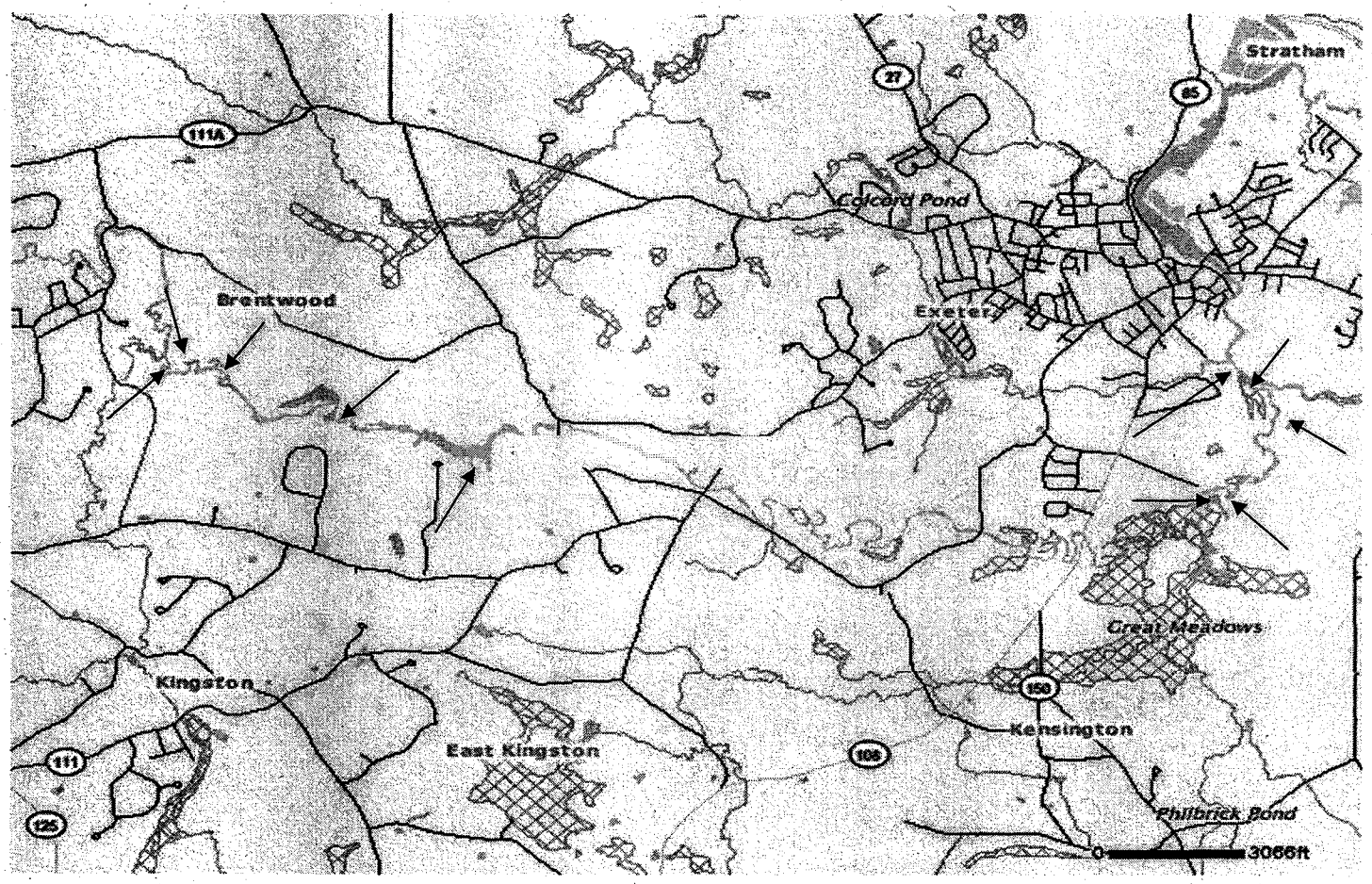

Figure I.2. Map of approximate seining sites, labeled with an arrow, sampled from August-October 2004 to recapture juvenile shad released into the Exeter River, NH.

Results

Environmental impact on egg hatch

The higher oxygen treatments (40%, 60%, and 80%) increased approximately 10% over the course of 12 hours, whereas the lower treatments (10% and 20%) increased approximately 20%. The 100% dissolved oxygen saturation treatment had a mean percent egg viability of 98.3% ± 1.7 and was significant from all other treatments (10, 20, 40, 60, and 80%), which all had a mean percent viability of 0% (Table I.1). Over the course of 24 hours, pH increased approximately one unit in each of the experimental treatments. Viability in a pH level of 4 (0%) was significantly different than in levels of 5 (78.3 ± 3.3%), 6 (63.3 ± 15.9%), or 7 (78.3 ± 10.1%) (Table I.1). There was a statistical difference between viability in salinities of 5 ppt (43.3 ± 10.9%) and 15 ppt (96.7 ± 3.3%). Egg viability in all other levels, 0 ppt (78.3 ± 1.7%), 10 ppt (86.7 ± 10.9%), 20 ppt (91.7 ± 1.7%), and 30 ppt (58.3 ± 20.3%), were not statistically different (Table I.1). There was no significant impact of nitrates or phosphates on egg hatch. Nitrate levels of 0, 14.59, and 29.18 mg l-1 resulted in 100 ± 0% egg viability, 3.65 mg l-1 resulted in 86.7 ± 4.4% viability, and 58.35 mg l-1 resulted in 85.0 ± 7.6% viability (Table I.1). Viability in phosphates of 0 and 0.04 (85.0 ± 2.9%), 2.08 (81.7 ± 3.3%), 4.17 (78.3 ± 3.3%), and 8.33 mg l-1 (80.0 ± 2.9%) were not significantly different (Table I.1).

Environmental impacts on larval survival

Larval survival in a dissolved oxygen saturation of 20% (0%) was significantly lower than all other levels. The remaining levels, 40% (55.0 ± 7.6%), 60% (65.0 ± 2.9%), and 100% (85.0 ± 7.6%), resulted in larval survival that was not significantly different (Table I.2). Survival in a pH of 7 (55.0 ± 0%) was significantly higher than in all other levels, and survival in pH 6 (25.0 ± 8.7%) was significantly higher than in pH 4 (0%) and 5 (0%) (Table I.2). There was an apparent peak in survival at a salinity of 10 ppt (65.0 ± 5.0%), with a gradual decline in survival as salinity increased or decreased. Survival in a salinity of 5 ppt (55.0 ± 0%) was not significantly different from 10 or 15 ppt (33.3 ± 7.3%) but was significantly higher than 0 (1.7 ± 1.7%), 20 (16.7 ± 6.7%), and 30 ppt (0 ± 0%) (Table I.2). There was no significant impact of nitrates or phosphates on larval survival. Survival in nitrates of 0 (85 ± 7.6%), 29.18 (68.3 ± 3.3%), and 58.35 mg l-1 (71.7 ± 4.4%) were not significantly different. Survival of larvae in phosphates of 0 (85.0 ± 7.6%), 4.17 (71.7 ± 1.7%), and 8.33 mg l-1 (76.7 ± 3.3%) were not significantly different.

Dissolved oxygen impacts on juvenile growth and survival

The results of the chronic study with 21 day post hatch (dph) juveniles showed that 20% (42.5 ± 11.8%) oxygen saturation resulted in significantly lower juvenile survival than all other treatments. The survivals in 40% (90.0 ± 5.8%), 60% (90.0 ± 6.1%), and 100% (96.7 ± 3.3%) dissolved oxygen treatments were

not significantly different from each other (Figure I.3). With an abrupt oxygen decrease, the 20% (10.0 ± 5.8%) oxygen saturation resulted in significantly lower survival than the 40% (72.5 ± 5.2%) and 100% (93.3 ± 6.7%) saturations, which were not significantly different from each other (Figure I.4). With the older juveniles (120 dph), there was a significant difference in survival among all treatments: 12% (3.3 ± 3.3%), 18% (50.0 ± 10.0%), and 100% (96.7 ± 3.3%) (Figure I.5). In the growth study, fish reared at 100% (16.6 ± 0.3 mm) oxygen saturation appeared larger by day 12 of the study than fish raised at 40% (15.8 ± 0.3 mm) saturation; however, these results were not significant (p=0.102). Fish in the 60% (16.1 ± 0.2 mm) oxygen saturation treatment had a mean length between the 40% and 100% treatments, but growth was not statistically different from either of the other two treatments (Figure I.6).

Impact of dams on emigration of shad juveniles

Fifty three seine hauls were conducted between 25 August and 25 October 2004 (Table I.3). All juvenile American shad were captured in the reach upstream of Pickpocket Dam (Figure I.1). Oxytetracycline marks were present on 19 of the 44 otolith pairs examined. Lengths ranged from 52 to 81 mm (Table I.4) with a mean of 65.0 ± 8.0 mm. Water quality data were collected from 16 August through 30 October. Oxygen saturation reached the 40% level for daily minima on 5 days in early September (Figure I.7).

Table I.1. Summary of viability results for shad egg hatch investigations conducted in the laboratory during 2005. Eggs were placed in glass beakers with aeration and subjected to varying environmental conditions.

Parameter	Level	Survival (%)*
Dissolved Oxygen (%)	10	0[b]
	20	0[b]
	40	0[b]
	60	0[b]
	80	0[b]
	100	98.3 ± 1.7[a]
pH	4	0[b]
	5	78.3 ± 3.3[a]
	6	63.3 ± 15.9[a]
	7	78.3 ± 10.1[a]
Salinity (‰)	0	78.3 ± 1.7[ab]
	5	43.3 ± 10.9[b]
	10	86.7 ± 10.9[ab]
	15	96.7 ± 3.3[a]
	20	91.7 ± 1.7[ab]
	30	58.3 ± 20.3[ab]
Nitrates (mg l-1)	0	100[a]
	3.65	86.7 ± 4.4[a]
	14.59	100[a]
	29.18	100[a]
	58.35	85.0 ± 7.6[a]
Phosphates (mg l-1)	0	85.0 ± 2.9[a]
	0.04	85.0 ± 2.9[a]
	2.08	81.7 ± 3.3[a]
	4.17	78.3 ± 3.3[a]
	8.33	80.0 ± 2.9[a]

* Significance group within parameter

Table I.2. Summary of results for shad larval survival investigations conducted in the laboratory during 2005. Larvae were placed in blue plastic beakers with aeration and subjected to varying environmental conditions.

Parameter	Level	Survival (%)*
Dissolved Oxygen (%)	20	0^{b}
	40	55.0 ± 7.6^{a}
	60	65.0 ± 2.9^{a}
	100	85.0 ± 7.6^{a}
pH	4	0^{c}
	5	0^{c}
	6	25.0 ± 8.7^{b}
	7	55.0 ± 0^{a}
Salinity (‰)	0	1.7 ± 1.7^{d}
	5	55.0 ± 0^{ab}
	10	65.0 ± 5.0^{a}
	15	33.3 ± 7.3^{bc}
	20	16.7 ± 6.7^{c}
	30	0^{d}
Nitrates (mg l-1)	0	85.0 ± 7.6^{a}
	29.18	68.3 ± 3.3^{a}
	58.35	71.7 ± 4.4^{a}
Phosphates (mg l-1)	0	85.0 ± 7.6^{a}
	4.17	71.7 ± 1.7^{a}
	8.33	76.7 ± 3.3^{a}

* Significance group within parameter

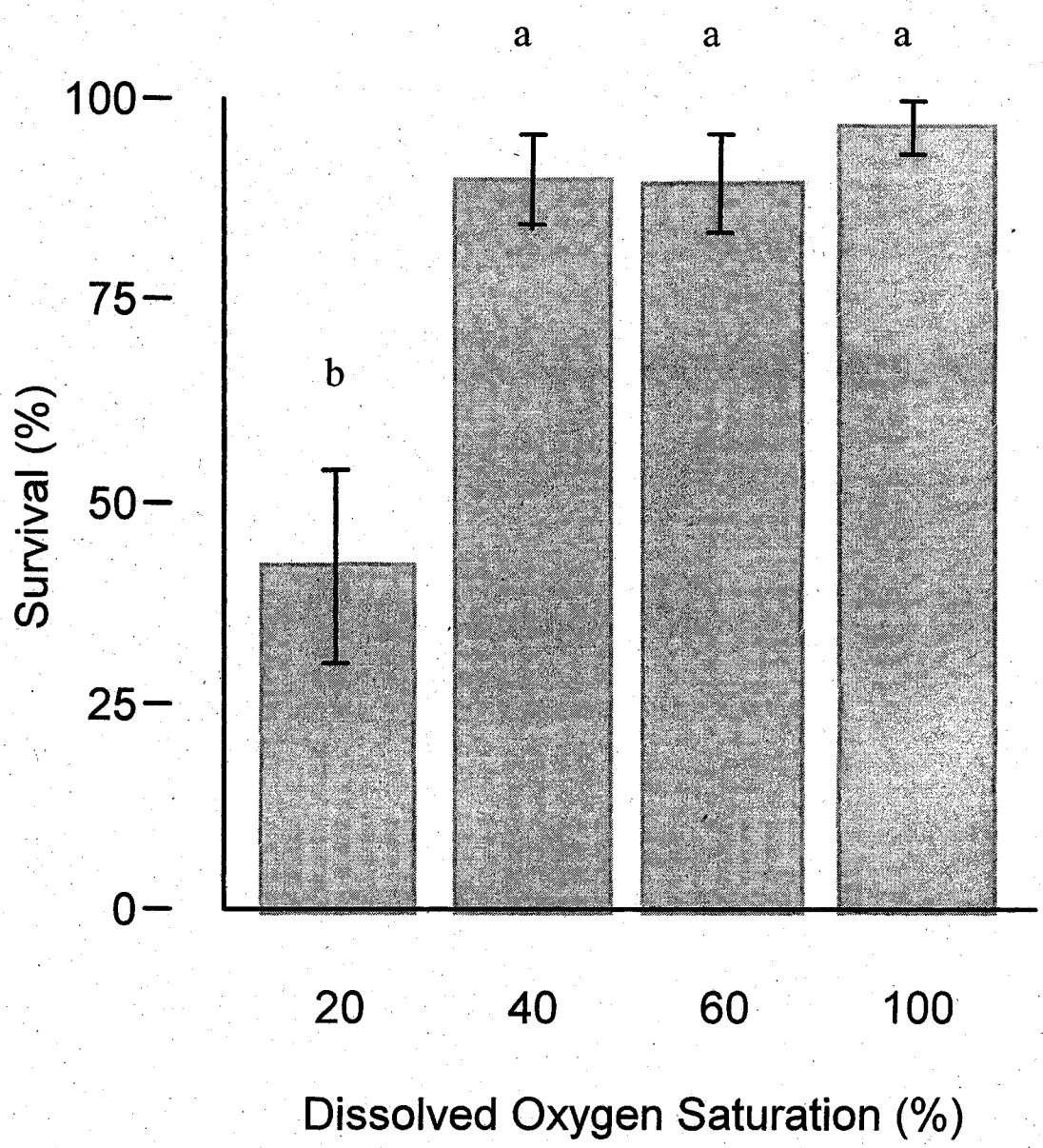

Figure I.3. Dissolved oxygen study on 21dph shad by the slow, continuous addition of nitrogen. Mean survival and standard error were calculated after start of nitrogen into experimental tanks.

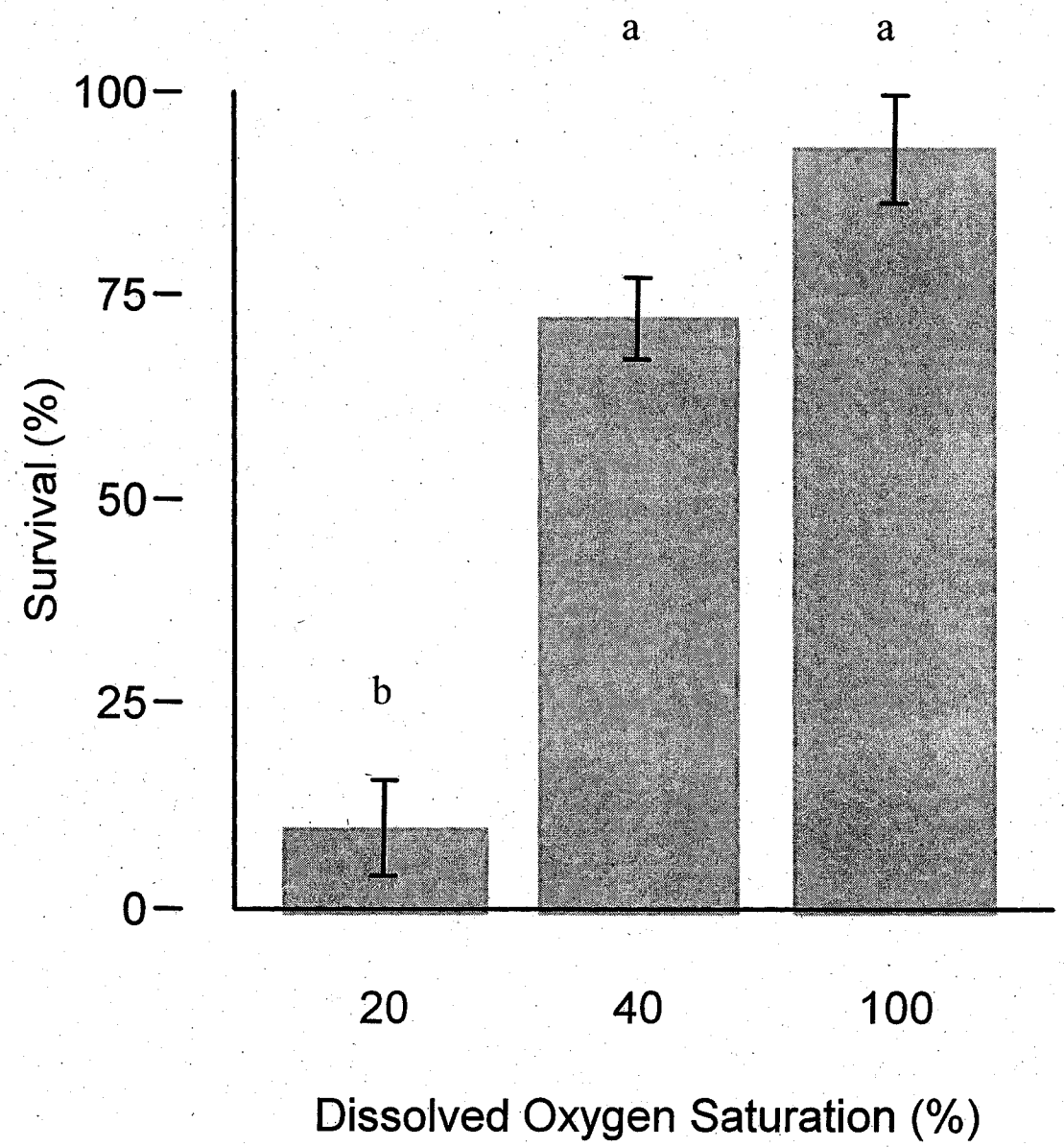

Figure I.4. Dissolved oxygen study on 21dph shad by the abrupt addition of nitrogen. Mean survival and standard error were calculated after start of nitrogen into experimental tanks.

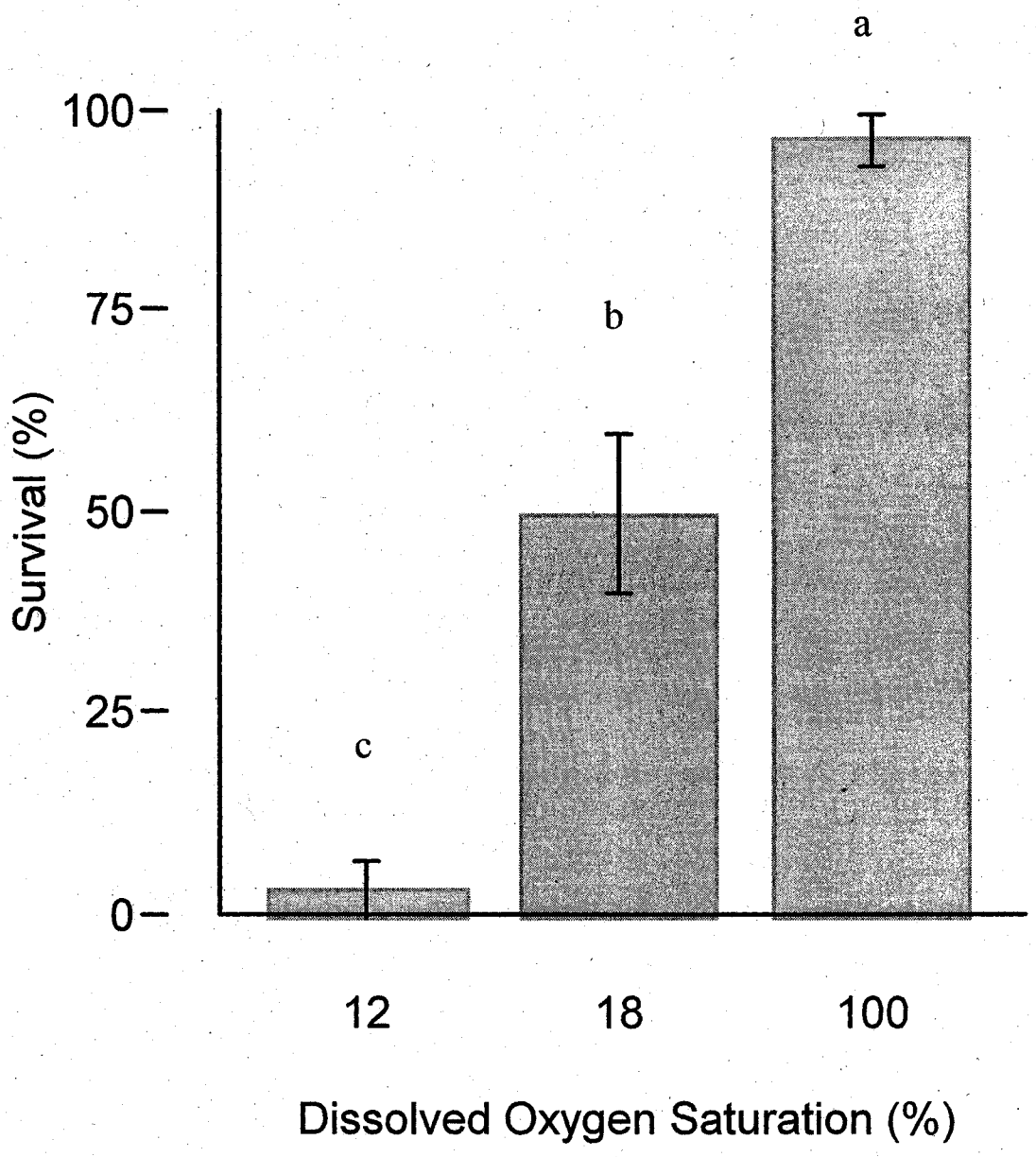

Figure I.5. Dissolved oxygen study on 120dph juvenile shad. Mean survival and standard error were calculated after start of nitrogen into experimental tanks.

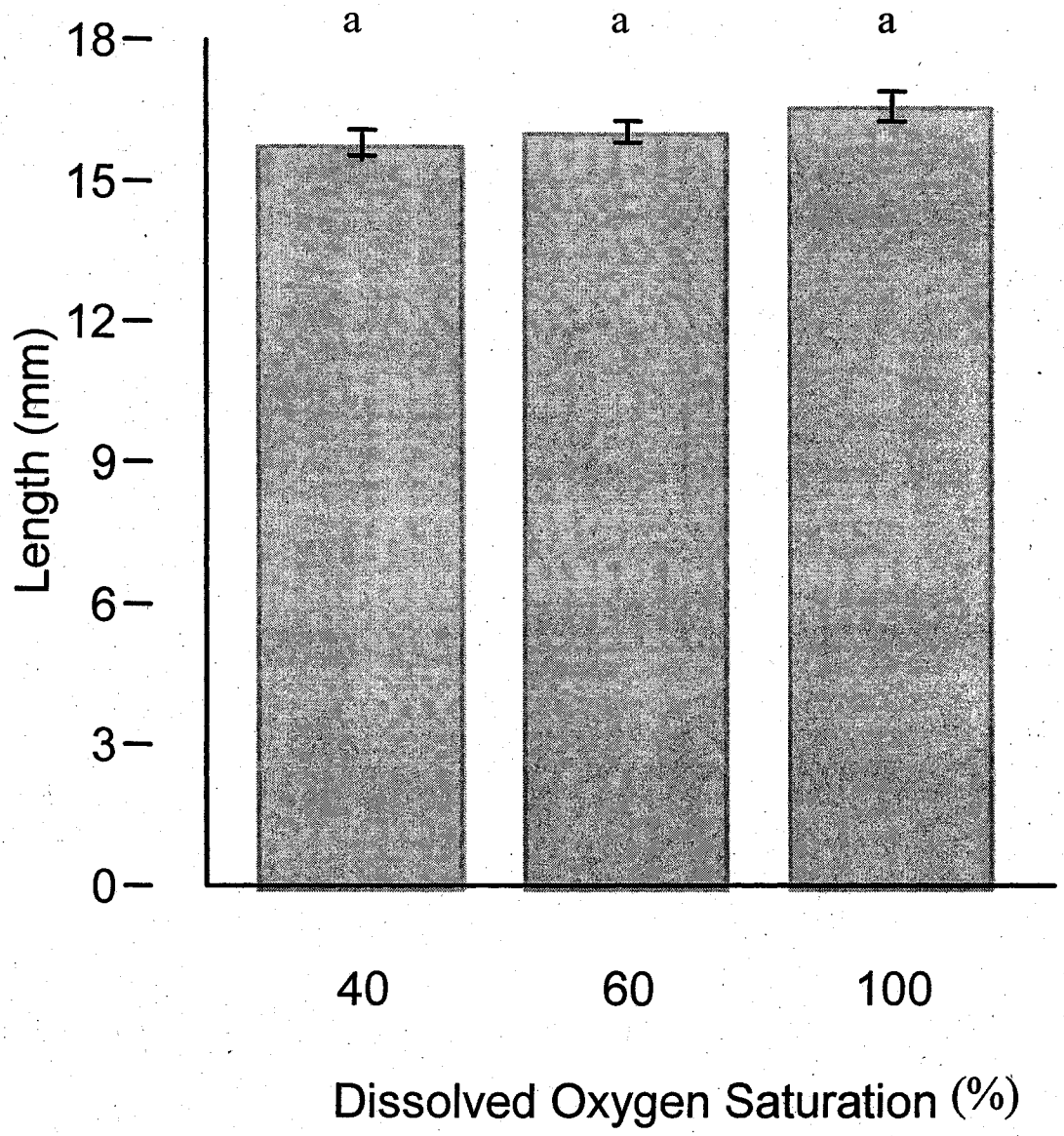

Figure I.6. Growth study on 28 dph shad subjected to varying levels of dissolved oxygen. Mean length for each treatment was found on day 12 and standard errors calculated.

Table I.3. Summary of seining results for American shad investigations conducted in the Exeter River, NH during 2004.

Date	# of Seine Hauls	# American shad caught	# American shad marked
	upper reach		
8/13/2004	3	0	N/A
8/25/2004	5	2	1
9/10/2004	5	38	14
9/24/2004	5	4	4
10/8/2004	5	0	N/A
10/25/2004	6	0	N/A
	lower reach		
8/25/2004	4	0	N/A
9/10/2004	5	0	N/A
9/24/2004	7	0	N/A
10/8/2004	6	0	N/A
10/25/2004	5	0	N/A
Totals	53	44	19

Table I.4. Lengths of American shad captured during investigations conducted in the Exeter River, NH during 2004.

Date	Haul #	Mean (mm)	Count
8/25/05	3	55.0	2
9/10/05	1	57.6	14
9/10/05	3	65.0	1
9/10/05	4	74.0	1
9/10/05	5	69.5	22
9/24/05	1	64.0	4

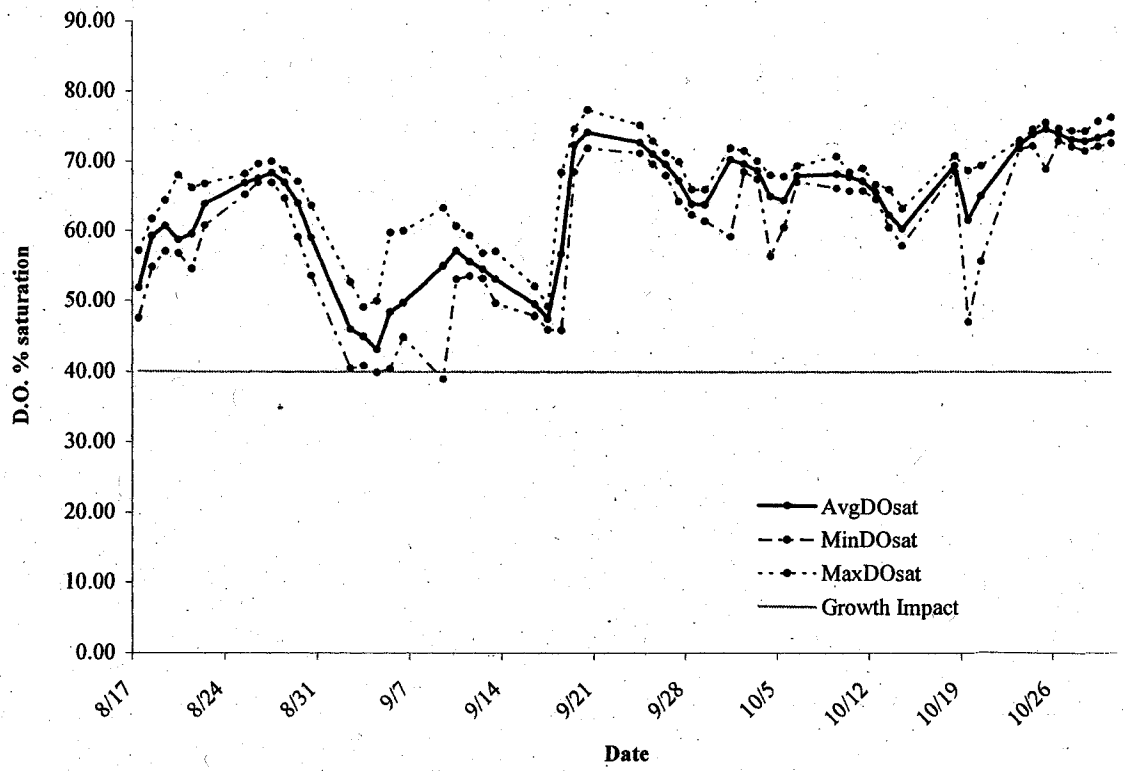

Figure I.7. Daily mean, maximum, and minimum dissolved oxygen saturation in the Exeter River, NH 2004, from YSI deployment.

Discussion

Laboratory Studies

Oxygen availability is an important factor in aquatic ecosystems. Because oxygen levels can be affected positively or negatively by other environmental conditions, fish must be able to adapt to changes in the oxygen levels by adapting respiratory and metabolic demands (Nikinmaa and Salama 1998). Of the abiotic factors tested in these laboratory experiments, depressed oxygen had the greatest effect on American shad egg hatch in the laboratory. This study indicates that shad eggs would not survive sustained dissolved oxygen levels of 80% (6.9 mg l-1) or less. Bradford et al. (1968), however, found that a good hatch of normal larvae (84% hatch) could occur in dissolved oxygen values as low as 4 mg l-1. One possible cause for this discrepancy was the constant movement of water by submersible pumps that occurred in the study by Bradford et al. (1968). The current study did not circulate water except for the two inputs of gaseous nitrogen. Shad eggs, based on the current study, require a higher dissolved oxygen saturation than other species including Atlantic salmon, whitefish, and vendace (Hamor and Garside 1977; Czerkies et al. 2002). This difference may be explained by a temperature difference between the species; the shad in this study were raised at a much higher temperature than the other fish. It has been shown that because embryos at higher temperatures grow faster, depressed oxygen is more of a limiting factor

than in fish that are reared in colder temperatures and develop more slowly (Hamor and Garside 1977).

Additionally, oxygen levels below 20% (1.7 mg l-1) resulted in mortality of early yolk-sac and 21 dph larvae. A dissolved oxygen of 40% (3.3 mg l-1) resulted in a lower average larval (21 dph) survival than the 100% (8.3 mg l-1), although it was not significant ($p=0.075$). This confirms Burdick's (1954) findings that shad could tolerate levels as low as 4 mg l-1, despite the previous prediction of 5 mg l-1 used by the Atlantic States Marine Fisheries Commission. There was a reduction in survival in larger juveniles (120 dph) at levels of 12% (1.1 mg l-1) and 18% (1.7 mg l-1) dissolved oxygen saturation. Even though the mean length of the 120 dph fish was 55.5 mm, mortality occurred at a mean dissolved oxygen similar to that of 83 mm juveniles studied by Chittenden (1973), which occurred at 1.3 mg l-1. This increased resistance to low dissolved oxygen as the shad gets older may be an effect of their method of respiration. In chinook salmon, newly hatched alevins respire primarily cutaneously; however, as the fish matures the gills become the primary mode of respiration. Respiration at hatch occurs across the gill filaments, in which blood flow is at a right angle to water flow. Respiration becomes more efficient as the fish matures with the development of gill lamellae, in which blood flow is countercurrent to water flow (Rombough and Ure 1991). Oxygen requirements vary widely among species; carp for instance have relatively low oxygen requirements. Carp larvae (3 dph) reared at 33°C did not reach a critical oxygen level until a 23% average (1.7 mg l-

1), when the larvae experienced a decrease in food uptake, ventilation difficulties, and lower growth rates (Wozniewski 1993). These carp, however, still had a survival of 88.5%. Wozniewski (1993) attributes this tolerance to the carp's ability to utilize atmospheric air. This use of atmospheric air is known as aquatic surface respiration and is seen in several fishes, including the mummichog (*Fundulus heteroclitus*), at low dissolved oxygen concentrations (Stierhoff et al. 2003).

In the current study, all survival thresholds for dissolved oxygen, with the exception of egg survival, are above the Fishery Management Plan recommendations for American shad [less than 5 mg l-1 (ASMFC 1985)]. This suggests that this standard is appropriate for all life stages except eggs, for which further examination is required. Minimum daily oxygen levels in the Exeter Dam impoundment have been shown to reach the ASMFC standard level in previous years (Langan and Jones 1996), including the relatively high flow year of 2004 when 40% dissolved oxygen (~4 mg l-1) was recorded. This indicates that drifting eggs and emigrating juvenile American shad can, and do, encounter impoundments with potentially detrimental oxygen levels. Therefore, efforts should be made to prevent prolonged exposure of American shad young-of-the-year to poorly oxygenated waters. Based on these results, any egg, larval, or gravid adult restocking efforts should be avoided in regions where oxygen levels may be depressed until water flow and water quality conditions can be considerably improved.

Variation in growth of juvenile shad was not significant among dissolved oxygen treatments, although there was a trend towards higher growth with increasing dissolved oxygen saturations. In some species growth and dissolved oxygen saturation were significantly correlated. For example, tilapia (*Oreochromis aureus*) displayed a positive correlation between growth rate and dissolved oxygen saturation up to a certain level (Papoutsoglou and Tziha 1996). As oxygen levels did not affect growth rate, it is possible that the lower oxygen threshold was not approached or a longer exposure time may have been necessary.

A low pH may also cause detrimental physiological effects on fish. Depressed pH has become an increasing problem with acid rain, nitrification from fertilizers, and industrial waste runoff (Hendrey 1987; Mason 1989; Stumm and Morgan 1996). This study found a pH of 4 resulted in a lower viability of eggs than pHs of 5, 6, or 7. Leim (1924) examined shad egg hatching in pH levels from 6 to 10.0+ at two temperatures (12ºC and 17ºC) and found hatch was only affected at a pH of 6 at 12ºC and levels over 10 at both temperatures. The discrepancy between the current study and that of Leim (1924) may be due to an interaction between temperature and pH. The current study was conducted at 21 ± 1°C, much higher than Leim's study. The tolerance level of only pH 6 that Leim reported at 12ºC may be due to the shad eggs optimal temperature range. Spawning usually does not start until 12ºC is obtained, but the average spawning temperature is between 16ºC and 17ºC (Massman 1952). Spawning can occur

at temperatures as high as those used in the current study, for example peak spawning in the Connecticut River in 1967 occured at 22°C (Marcy 1976). The present results are also fairly consistent with the tolerance observed for Atlantic salmon embryos, which at their early stages had a lower lethal pH limit of 3.6 (Daye and Garside 1977).

The sensitivity of larvae to pH was much greater than that of the embryos both in the current study and in Atlantic salmon (Daye and Garside 1977). This may be due to the protection of the zona radiata and perivitelline fluid of the encapsulated embryo (Daye and Garside 1977). This study found that pH levels at or below 6 resulted in decreased larval shad survival, with most significant mortalities in pHs of 4 and 5. These result support a study by Leach and Houde (1999), who found that decreasing pH from 7 to 6 led to a reduction in larval survival, although the results were not significant. However, when Leach and Houde (1999) combined a low pH with low temperature or prey level, survival and production of larvae were severely impacted. Other species have shown similar tolerances to pH, such as salmon fry that will continue development to actively feeding fry at a pH greater than 5 (Lacroix et al. 1985). Due to the susceptibility of shad to changes in or low pH, areas around agriculture, stormwater, or mine runoff where there may be a low pH as well as periods after heavy rains should be avoided for shad restoration efforts.

Anadromous fish face a unique osmotic challenge, because they have to adapt to both fresh and salt water environments. Eggs are normally deposited in

freshwater; however, in areas where an impassable dam is close to the mouth of a river, adults may be forced to spawn in tidally influenced areas. Leim (1924) found that shad egg hatch was unaffected by salinity until levels of 22.5 ppt, over which hatch decreased significantly. Salinities at or above this level did not significantly affect hatch in the current study. Shad egg tolerance to high salinity is an anomaly among anadromous fishes such as striped bass and rainbow smelt, which typically are susceptible to salinities above approximately 15 ppt (Winger and Lasier 1994, Ayer et al. 2005).

Both extreme high (30 ppt) and extreme low (0 ppt) salinities were detrimental to the survival of early larval shad in this study. This supports Leim's (1924) findings that larvae in freshwater were active for only one day after hatching, larvae at 7.5 ppt were normal and swam vigorously after hatch, larvae at 15 ppt were active for a slightly shorter period than the 7.5 ppt, and larvae at 22.5 ppt were abnormal at hatch and did not survive long. The high mortality in 0 ppt salinity, found both in the current study as well as that by Leim (1924), may be influenced by handling stress (Chittenden 1973). However, this handling stress would not be experienced naturally. Limburg and Ross (1995) found no difference in mortality rates between shad raised in 0 ppt and 20 ppt salinity. They hypothesize other factors, besides salinity, are driving the spawning migration upriver. Zydlewski and McCormick (1997a) concluded that shad could not physiologically tolerate salinities typical of full-strength seawater until after metamorphosis. The delayed tolerance to salinity has been shown to be linked

to metamorphosis, declining temperatures, and outmigration of euryhaline species by an increase in gill formation, chloride cell density, and Na+,K+-ATPase activity (Zydlewski and McCormick 1997a and b; Zydlewski and McCormick 2001; Ewing et al. 2001).

Different fish species have varying levels of tolerance to nitrates and phosphates, but no visible affect on survival of shad eggs or larvae was seen in this study up to levels of 58.35 mg l-1 nitrate and 8.33 mg l-1 phosphate. Nitrates did not affect egg hatch in Chinook or coho (*Oncorhynchus kisutch*) salmon, but levels of 5-10 mg l-1 were found to be mildly toxic to early life stages of rainbow and steelhead trout (*Oncorhynchus mykiss*) (Kincheloe et al. 1979). Common carp hatched normally in phosphate levels up to 0.12 mg l-1, but levels above this reduced hatch and abnormalities in larvae were observed (Thoor et al. 1983). Many fish exhibit low toxic thresholds of nitrates and phosphates, similar to levels found in nature. However, two species of flatfish (*Limanda yokohamae* and *Paralichtys olivaceus*) were found to only be sensitive to levels of nitrates and phosphates between 100 and 1,000 mg l-1 (Yasunaga 1976). Nitrates and phosphates in aquatic ecosystems have increased with the application of fertilizers and construction of sewage treatment facilities along the shores of some waterways. Nitrate (as N) and phosphate (orthophosphate as P) levels in New Hampshire rivers have reached 0.317 mg l-1 and 0.7203 mg l-1 respectively (NHDES National Coastal Assessment Tidal Water Quality Monitoring Data). Determining what levels are harmful allows better monitoring in these areas;

however, more work should be done to elucidate levels that can cause endocrine disruption in adults resulting in decreased recruitment of offspring.

Field Studies

The first seine hauls to monitor juvenile American shad emigration on 13 August were unsuccessful at the Pickpocket Dam impoundment due to high water. In past years, juvenile shad have been captured at this location and therefore, it was a logical starting point for this project. This research was based, in part, on the evidence that in most years juvenile shad encounter dams with insufficient overtopping flow to allow them to pass. Abnormally high flows in 2004, however, alleviated this passage problem. Sampling juvenile shad trapped in impoundments was not a viable option and seine hauls were therefore conducted upstream of impoundments in areas likely to concentrate juvenile shad. Although seining did produce results, the total number of shad captured was low (N=44). Abnormally high water increased the number of locations available for juvenile shad to seek refuge and many of these areas were inaccessible to the boat and seine because of vegetation and debris.

All juvenile shad were encountered in the river reach upstream of the Pickpocket dam. It is difficult to draw firm conclusions for the absence of juvenile shad in the lower reach seines but possible explanations can be offered. Juvenile shad emigration may have occurred very quickly due to the high water flows. Therefore, bi-weekly seining may not have been temporally intensive

enough to capture shad as they traversed the lower reach. Differences in river morphology between the upper and lower reaches may have played a role in the lack of catch downstream as well. In the upper reach, fish were found in eddies associated with river bends. The wider, slower moving character of the lower reach did not contain as many of these areas as the upper section. If the pattern is not an effect of sampling bias, it implies decreased survival of downstream emigrants passing over the Pickpocket Dam.

The timing of capture of juveniles during 3 seining inventories from the upper reach of the Exeter River (25 August –24 September) is suggestive of emigration. Caution is warranted in this interpretation, however, due to the low sample size. It should also be noted that although most seine sites did overlap from one sampling event to the next, exact replication could not be maintained. One result in support of the documentation of emigration is the difference in mean size between catches in upstream versus downstream sections of the upper reach. Size has been shown to play a role in the timing of downstream movement of juvenile American shad, with the larger individuals emigrating prior to the smaller ones (Limberg 1996). The mean size of the 14 juvenile shad captured farthest upstream on 10 September was significantly smaller than the 22 fish captured downstream ($P < 0.0001$).

The laboratory and field studies suggest emigrating American shad released in the upper reaches of the Exeter River will have a decreased probability of reaching adulthood, whether it be from impassible dams with no

overflow or poor water quality behind dams. To improve American shad restoration in the Exeter River, survival during outmigration must increase by improving water quality and downstream fish passage. In addition, upstream migration of the adults through the fishways should be monitored in order to ensure that they can travel far enough upstream to spawn, so progeny are not prematurely exposed to saline waters.

CHAPTER II

RAINBOW SMELT (*OSMERUS MORDAX*)

Abstract

Recruitment of smelt has steadily decreased over the last few decades, possibly due to the construction of physical impediments such as dams and an increase in anthropogenic pollution. The effects of dissolved oxygen (DO), pH, salinity, nitrate, and phosphate levels on hatch and survival of larval rainbow smelt were examined in a closed laboratory system. In addition, the viability of eggs incubated in the Winnicut and Squamscott Rivers, New Hampshire was compared. Extreme levels of dissolved oxygen (10%, 1.09 mg l-1), pH (4), and salinity (20-30 ppt) negatively affected egg hatch, whereas nitrates and phosphates had no significant impact. Larval survival was reduced by pHs at or below 5, but all other parameters (DO, salinity, nitrates, and phosphates) had no detrimental effects on larval survival at levels tested. In general, the eggs placed in the siltier Winnicut River had a lower viability than those incubated in the Squamscott River that had a greater incidence of fungal coverage. These results can be used to improve restocking efforts currently underway throughout the Northeast.

Introduction

Rainbow smelt (*Osmerus mordax*) are one of the most important fish found in New Hampshire waters. Smelt support an important recreational winter fishery, as well as an important part of the ecology in the rivers, estuaries, and ocean through which they migrate. Historically, rainbow smelt were found from Labrador to New Jersey, but significant populations have been reduced to Massachusetts northward (Bigelow and Schroeder 1953; Murawski and Cole 1978).

Humans have negatively impacted the aquatic environment in many ways, causing declines in fish populations. Anadromous fish are especially vulnerable to impacts by humans due to their migration through many different environments. When they enter freshwater rivers to spawn, they are often negatively impacted by physical impediments, like dams, and pollutants, imposed by the surrounding human populations (Rothschild 1961; Jackson 1952). Many rivers have seen a decline in rainbow smelt recruitment, which may be due to the unfavorable environmental conditions to which eggs are exposed. Levels of industrial activity have been closely linked to fluctuations in smelt populations. For instance, in the years of the Great Depression (1929-1941), smelt populations increased with the closing of many industrial plants along the tributaries of Great Bay, New Hampshire. Smelt populations, however, plummeted prior to, and during World War II because of increased industrial

activity (Jackson 1952) and high harvest levels (NH Fish and Game 1982). Despite the importance of rainbow smelt to New Hampshire's fisheries and their severe population declines, restoration efforts have not kept pace with those for other anadromous species (NH Fish and Game 1982).

The decrease in smelt populations over the last several decades may be due, in part, to a decreased hatch rate (NH Fish and Game 2004). Several experiments have been conducted to better understand the physical restrictions and environmental conditions affecting egg hatch. Physical obstructions blocking upstream migrations can cause an overcrowding of eggs, which is associated with heavy fungal growth that prevents high hatch (McKenzie 1947). Not only do dams restrict access to desirable spawning areas and cause overcrowding, they also allow the tidal deposition of large amounts of silt, which may smother the eggs (Jackson 1952). Also, a positive trend was found between the distance of egg deposition upstream and percent hatch (Rothschild 1961), which suggests that smelt eggs must be further upstream than some dams allow in order to maximize hatch. Furthermore, smelt eggs are very sensitive to relatively high salinity levels that may be present at available spawning sites below dams (Ayer et al. 2005).

Pollution and environmental changes influenced by human impacts have caused detrimental changes to the spawning habitat as well. An increase in periphytic algae has been observed in tributaries where smelt populations have been declining; however, no statistical correlation was found between the

presence of algae and egg hatch in a study by Lapierre et al. (1999). Eutrophying compounds, such as nitrates, may cause this increase in algal growth and also impact hatching of fish eggs. In addition, low pH impaired hatching of rainbow smelt if exposure was prior to the eyed embryo stage (Geffen 1990). Despite efforts to find a cause of decreased egg hatch, smelt populations continue to decline. Lethal and damaging levels of dissolved oxygen, pH, salinity, nitrates, and phosphates that can influence smelt egg hatch need to be defined in order to successfully evaluate available spawning habitat.

Materials and Methods

Environmental impact on egg hatch and early larval survival

In March 2005 and 2006, adult smelt were collected from the Squamscott River, Exeter, NH by recreational ice fishermen and housed in the Aquaculture Research Center at the University of New Hampshire, Durham, New Hampshire. Sexually mature rainbow smelt were also captured from the Salmon Falls River, Dover, NH using a fyke net. In April 2005 and 2006, eggs and larvae were also obtained from the Damariscotta River, Damariscotta, Maine. The smelt were anesthetized with tricaine methanesulfonate (100 mg l-1 Tricaine-S; Western Chemicals, Ferndale, WA), blotted dry on the ventral side, and strip spawned by methods described by Ayer et al. (2005). Eggs were expressed into a beaker by applying pressure to the abdomen of the females. Milt was expelled in the same fashion into small vials. Milt from several males was added to the eggs of a single female and activated with sterile water. Eggs were incubated in batches for approximately 20 days or until hatch with supplemental aeration in MacDonald jars in a 10°C cold room at the University of New Hampshire during March-June 2005 and 2006. The eggs that were not used in hatch experiments were incubated in aerated well water in MacDonald jars until hatch.

Egg viability was quantified by raising twenty, eight day post fertilization (dpf) eggs in 1 l beakers with 800 ml of non-chlorinated freshwater at 10°C until hatch, which began at 17 dpf. Early larval survival studies were conducted under

the same water and temperature regimes as the hatch study; however, twenty, one day post hatch (dph) larvae were tested for three days. Larvae were not fed during this period, because they still had endogenous yolk reserves. Hatch and larval survival were tested under varying dissolved oxygen, pH, salinity, nitrate, and phosphate levels in triplicate. Dissolved oxygen of levels 10% (1.1 mg l-1), 20% (2.2 mg l-1), 40% (4.4 mg l-1), 60% (6.6 mg l-1), and 100% (10.9 mg l-1, control) were obtained by dispensing gaseous nitrogen into the beakers. Because the experimental dissolved oxygen levels rose approximately 1 mg l-1 over the course of 12 h, dissolved oxygen was monitored with a Handy Gamma meter (OxyGuard International, Birkerød, Danmark) and adjusted twice daily by adding gaseous nitrogen. Sulfuric acid was added dropwise into beakers until pH levels of 4, 4.5, 5, 5.5, 6, and 7 (control) were obtained and validated with a pH meter PHAST-CHEK Pocket (VWR, West Chester, PA). After 24 h, levels increased by approximately one unit, so pH was adjusted daily by the addition of sulfuric acid. Salinities of 0 (control), 5, 10, 15, 20, and 30 ppt were reached by the addition of Instant Ocean™ (Mentor, Ohio) and validated by using a refractometer (Spartan Refractometers, Tokyo, Japan). Nitrate concentrations of 0 (control), 0.73, 3.65, 7.30, 14.59, and 29.18 mg l-1 nitrate were tested by the dissolution of sodium nitrate (Sigma, St. Louis, MO). Phosphates concentrations of 0 (control), 0.04, 0.21, 0.42, 2.08, and 4.17 mg l-1 phosphate were tested by the dissolution of potassium phosphate dibasic trihydrate (Sigma, St. Louis, MO). Viability of the eggs was determined just prior to hatch by visual examination.

Viable eggs are transparent, whereas nonviable eggs are yellow or white and opaque. Survival of larvae was calculated on day three of the experiment. Percent data were arcsine square root transformed and analyzed by ANOVA ($p<0.05$) using JMP Software (SAS Institute Inc., Cary, NC). In addition, a Tukey-Kramer post hoc test was performed to display significant differences among treatments.

Field comparison of Squamscott and Winnicut Rivers

In order to compare hatching success in different rivers, eight incubators were placed in both the Squamscott and Winnicut Rivers, which are tributaries of Great Bay, NH. These incubators were made of round (15.2 cm diameter) concrete slabs (6.4 cm thick) with 4 bolts poured into them (Figure II.1). Slate plates (10.2 x 10.2 cm) were drilled to fit onto the bolts. Four of the eight incubators were covered by a metal mesh screen (mesh size 1.3 mm) to determine if predation influenced egg hatch. The incubators were held in place by a 1.2 m piece of steel reinforcing bar placed through an eye bolt poured in the concrete. Approximately 100-700 eggs, strip spawned as in the laboratory studies, were placed by polypropylene transfer pipettes onto the slate plates. The plates were submerged in water from their designated river and transported with supplemental aeration to the sampling sites. The incubators were placed into the river and the plates transferred directly from the cooler to the incubator. Control plates were placed in aquaria with a Resun SP-800 submersible pump

(Guangdong Risheng Group Co., Ltd., Guangdong, China) and air stone in the laboratory at 5°C in one of three conditions: Winnicut River water, Squamscott River water, and non-chlorinated well water. The study was run in triplicate with the same batch of eggs and for the same length of time as the plates in the rivers. Pictures of the plates were taken in situ by a Pentax OptioWPi (Pentax of America Inc., Golden, CO) in order to quantify viability of the eggs based on the color and clarity of the eggs. Percent viability was calculated for days 1 and 10, and from this a percent change in viability was determined.

The percent change in viability was arcsine square root transformed and an ANOVA ($p<0.05$) was performed using JMP software (SAS Institute Inc., Cary, NC). A Tukey-Kramer post hoc test was executed to determine significances between treatments.

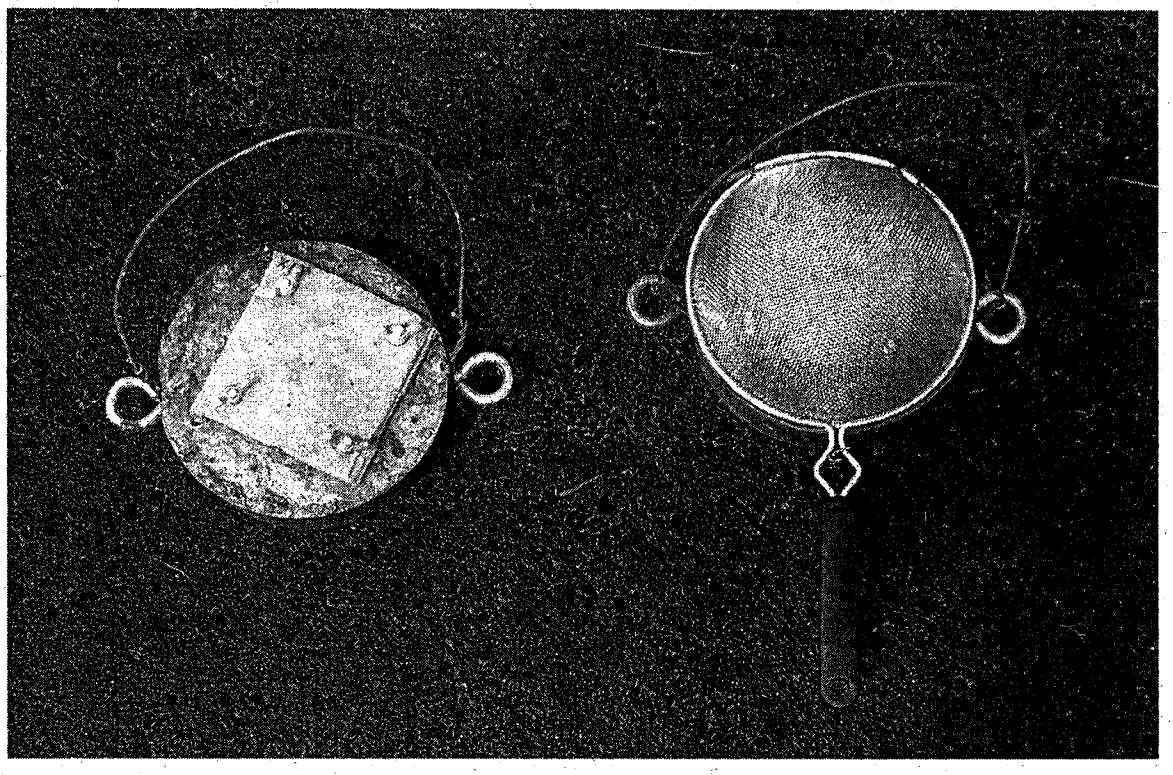

Figure II.1. Pictures of incubators, both uncovered (left) and covered (right), placed into the Winnicut and Squamscott Rivers.

Results

Environmental impact on egg hatch

The eggs in 20, 40, 60, and 100% dissolved oxygen saturation treatments were all viable (100%) and were significantly higher than those in 10% saturation, which had no survival (0%) (Table II.1). Viability in pH levels of 4.5 (98.3 ± 1.7%) 5, 5.5, 6, and 7 (100%) was significantly higher than viability at a pH level of 4 (13.3 ± 6.7%) (Table II.1). The viability of eggs at a 0, 5, 10, and 15 ppt (100%) salinity was statistically different than those at 20 and 30 ppt (0%) (Table II.1). There was no significant impact of nitrates or phosphates on egg hatch. Nitrate levels of 0, 0.73, 7.30, and 14.59 mg l-1 resulted in 98.3 ± 1.7% egg viability, while 3.65 and 29.18 mg l-1 resulted in 100% viability (Table II.1). Phosphates of 0.04 and 4.17 mg l-1 had 100% viability; 0, 0.42, and 2.08 mg l-1 had 98.3 ± 1.7% viability; and 0.21 mg l-1 had 95.0 ± 5.0% viability (Table II.1).

Environmental impacts on larval survival

Larval survival was not significantly impacted by a reduction in dissolved oxygen. All levels tested resulted in mean survival above 90%: 10% (93.3 ± 4.4%), 20% (96.7 ± 1.7%), 40% (95.0 ± 2.9%), 60% (100%) and 100% (100%) (Table II.2). Survivals in pHs of 5.5, 6, and 7 (100%) were significantly higher than those in all other levels, and survival in a pH 5 (80.0 ± 7.6%) was significantly higher than that in pHs of 4 (0%) and 4.5 (21.7 ± 6.0%) (Table II.2).

Salinities of 0 (100%), 5 (100%), 10 (98.3 ± 1.7%), 15 (93.3 ± 6.7%), 20 (98.3 ± 1.7%), and 30 ppt (98.3 ± 1.7%) did not have significantly different effects on survival (Table II.2). There was no significant impact of nitrates or phosphates on larval survival. Nitrates of 3.65, 7.30, and 14.59 mg l-1 resulted in a survival of 100%, while levels of 0 and 29.18 mg l-1 had a survival of 96.7 ± 3.3% and 0.73 mg l-1 had a survival of 98.3 ± 1.7% (Table II.2). Phosphates of 0, 0.04, 0.21, and 0.42 mg l-1 had survival of 100%, 2.08 mg l-1 had survival of 96.7 ± 1.7%, and 4.17 mg l-1 had survival of 90.0 ± 5.0% (Table II.2).

Field Comparison of Squamscott and Winnicut Rivers

The field experiments were ended after ten days due to the difficulty in assessing egg viability with silt and fungal cover. In general, the plates from the Winnicut River were observed to have high silt deposits, especially on the incubators that were covered with mesh (Figure II.2a). In the Squamscott River, the plates had high fungal growth, also with higher coverage found in the covered incubators (Figure II.2b). There appeared to be no differences in predation between the mesh covered and uncovered plates; therefore, only the uncovered plates were quantified for egg viability. The plates in the Winnicut River had a higher percent change (40.8 ± 6.3%) in viability than both those in the Squamscott River (17.2 ± 5.6%) and those in control (well) water (10.4 ± 2.6%). The viability of laboratory controls for both rivers, Winnicut (24.3 ± 2.8%) and

Squamscott (29.6 ± 4.8%), were not significantly different from any other group (Table II.3).

Table II.1. Summary of viability results for smelt egg hatch investigations conducted in the laboratory during 2005 and 2006. Strip spawned eggs were placed in glass beakers with aeration and subjected to varying environmental conditions.

Parameter	Level	Viability (%)*
Dissolved Oxygen (%)	10	0[b]
	20	100[a]
	40	100[a]
	60	100[a]
	100	100[a]
pH	4	13.3 ± 6.7[b]
	4.5	98.3 ± 1.7[a]
	5	100[a]
	5.5	100[a]
	6	100[a]
	7	100[a]
Salinity (‰)	0	100[a]
	5	100[a]
	10	100[a]
	15	100[a]
	20	0[b]
	30	0[b]
Nitrates (mg l-1)	0	98.3 ± 1.7[a]
	0.73	98.3 ± 1.7[a]
	3.65	100[a]
	7.30	98.3 ± 1.7[a]
	14.59	98.3 ± 1.7[a]
	29.18	100[a]
Phosphates (mg l-1)	0	98.3 ± 1.7[a]
	0.04	100[a]
	0.21	95.0 ± 5.0[a]
	0.42	98.3 ± 1.7[a]
	2.08	98.3 ± 1.7[a]
	4.17	100[a]

*** Significance group within parameter**

Table II.2. Summary of survival results for smelt larval investigations conducted in the laboratory during 2005 and 2006. Yolk-sac larvae were placed in glass beakers with aeration and subjected to varying environmental conditions for three days.

Parameter	Level	Survival (%)*
Dissolved Oxygen (%)	10	93.3 ± 4.4 [a]
	20	96.7 ± 1.7 [a]
	40	95.0 ± 2.9 [a]
	60	100 [a]
	100	100 [a]
pH	4	0 [d]
	4.5	21.7 ± 6.0 [c]
	5	80.0 ± 7.6 [b]
	5.5	100 [a]
	6	100 [a]
	7	100 [a]
Salinity (‰)	0	100 [a]
	5	100 [a]
	10	98.3 ± 1.7 [a]
	15	93.3 ± 6.7 [a]
	20	98.3 ± 1.7 [a]
	30	98.3 ± 1.7 [a]
Nitrates (mg l-1)	0	96.7 ± 3.3 [a]
	0.73	98.3 ± 1.7 [a]
	3.65	100 [a]
	7.30	100 [a]
	14.59	100 [a]
	29.18	96.7 ± 3.3 [a]
Phosphates (mg l-1)	0	100 [a]
	0.04	100 [a]
	0.21	100 [a]
	0.42	100 [a]
	2.08	96.7 ± 1.7 [a]
	4.17	90.0 ± 5.0 [a]

*** Significance group within parameter**

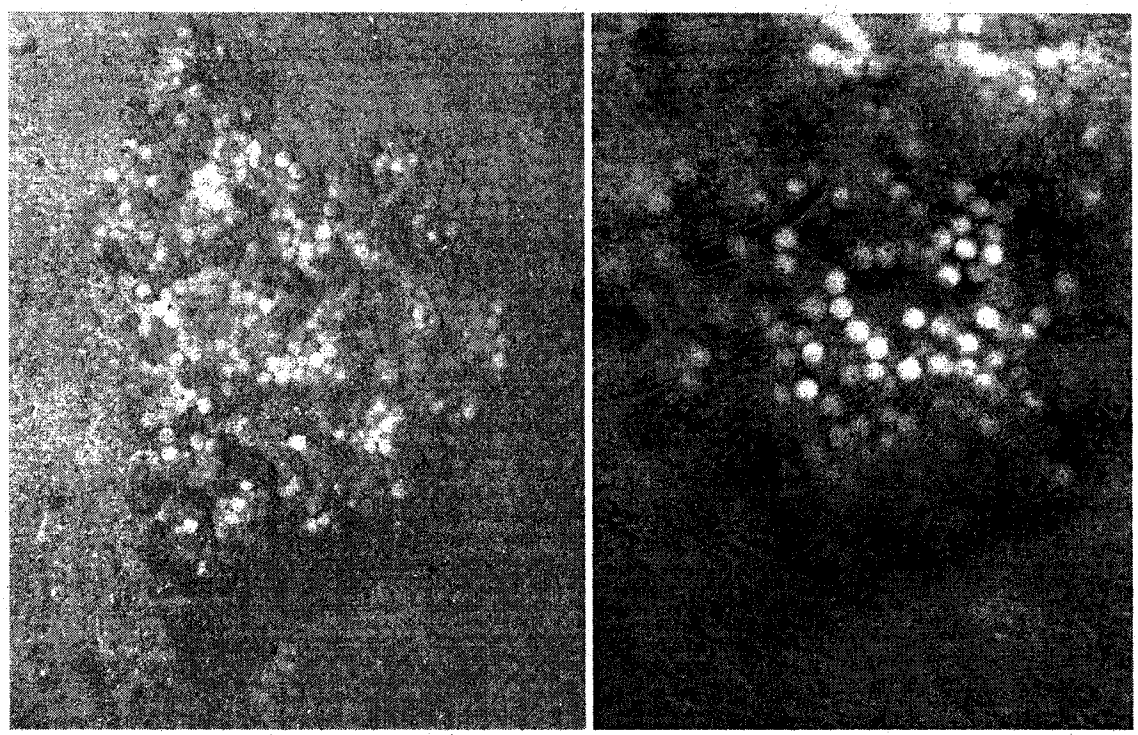

Figure II.2. Photograph of eggs on a slate plate placed in the siltier Winnicut River (a) and fungal laden Squamscott River (b) on day 10.

Table II.3. Summary of viability results for smelt investigations conducted in the Winnicut and Squamscott Rivers, NH during 2006. Strip spawned eggs were placed on slate plates and viability monitored for 10 days.

Treatment	Day 1 Mean Viability (%)	Day 10 Mean Viability (%)	Mean Change in Viability (%) *
Winnicut	78.7	37.9	40.8 [a]
Squamscott	57.6	40.4	17.2 [b]
Winnicut control	46.0	21.7	24.3 [ab]
Squamscott control	59.0	29.4	29.6 [ab]
ARC	100	89.6	10.4 [b]

*Significance group

Discussion

Laboratory Studies

Oxygen availability is a limiting factor in many aquatic ecosystems. Fish must be able to adapt respiratory and metabolic demands to changes in the dissolved oxygen levels (Nikinmaa and Salama 1998). Rainbow smelt eggs and larvae were found to be fairly hardy, with mortality occurring at extremely low levels of dissolved oxygen. Eggs were unaffected by dissolved oxygen until reduced to 10% (1.1 mg l-1) saturation. These results are similar to that of walleye (*Stizostedion vitreum*), which were shown to hatch in levels as low as 2 mg l-1 (Oseid and Smith 1971). The walleye in hypoxic conditions, however, were shown to have incubated longer and hatched at smaller lengths than those incubated in more highly oxygenated waters. A higher successful hatch in areas of high water flow, documented in a study by the Massachusetts Division of Marine Fisheries (2000), was thought to be due to increased oxygen. Because the present study showed that only extreme low dissolved oxygen causes a decrease in viability, it is likely that another factor, besides oxygen content, is responsible for the increased hatch success. Instead, it may be the low silt deposition, which can reduce oxygen availability to eggs even in oxygenated water. This may also explain the increase in hatch reported by Sutter (1980) in eggs that were deposited on aquatic vegetation versus those adhered on the river bottom. Silt deposition has also been shown to decrease survival of both

salmon and northern pike eggs (Shelton and Pollock 1966; Hassler 1970).

In the present study, dissolved oxygen levels as low as 10% (1.1 mg l-1) did not cause a reduction in yolk-sac larval survival; therefore, dissolved oxygen in the water does not appear to be a limiting factor in early larval survival. Larval smelt tolerance to low dissolved oxygen is comparable to that of the carp, which live in depressed oxygen environments. Carp larvae reared at 33°C did not reach a critical oxygen level until a 23% average saturation (1.7 mg l-1), when the larvae experienced a decrease in food uptake, respiratory difficulties, and decreased growth (Wozniewski 1993). These carp, however, still had a survival of 88.5%. One possible explanation for the similarity between the smelt larvae in this experiment and the carp larvae may be in the difficulty to maintain a dissolved oxygen saturation of 1.1 mg l-1. Nitrogen was bubbled in several times throughout the day, but levels occasionally rose above 2 mg l-1. However, these results are also similar to that of other anadromous species, which determined an LC_{50} of 1.6 mg l-1 and 1.8 mg l-1 for rainbow trout parr and adult common smelt (*Retropinna retropinna*), respectively (Landman et al. 2005). Based on the tolerance of eggs and larval smelt to depressed dissolved oxygen, factors such as siltation on eggs should be more influential when developing a management and restoration plan.

Because smelt spawn in the spring during periods of snow meltoff and high rains, their progeny are especially susceptible to low pH exposure. Depressed pH has become an increasing problem with acid rain, nitrification from

fertilizers, and industrial waste runoff (Hendrey 1987; Mason 1989; Stumm and Morgan 1996). In this study, smelt eggs were tolerant to lower levels of pH; however, larvae were less tolerant to depressed pH. Egg viability did not decrease until a pH of 4 was reached. Geffen (1990) also found that eggs after the eyed-embryo stage were not significantly affected by exposure to a pH of 4.5. However, eggs prior to this stage were more vulnerable to pH. This is similar to the pH tolerance of Atlantic salmon, in which a pH of 3.6 was lethal (Daye and Garside 1977). Larval tolerance of depressed pH was not as great as egg tolerance in the current study. Survival of larvae was significantly reduced when a pH of 5 was attained. This phenomenon has been seen in Atlantic salmon as well and was suggested to be due to the protection of the zona radiata and perivitelline fluid of the egg (Daye and Garside 1977). Because mortalities occur at low pH levels, pH needs to be considered in restoration attempts in areas where agricultural and mine runoff may be compounded by spring snowmelt and rain.

Anadromous fish face a unique osmotic challenge, as they have to adapt to both fresh and salt water environments. Results from this study indicated that salinities of 20 ppt or more were lethal to smelt eggs, which is in agreement with a study performed by Ayer et al. (2005). However, Akeilaszek et al. (1985) found that eggs could tolerate levels up to 24 ppt seawater. The current study on smelt is similar to hatch studies on striped bass (*Morone saxatilis*) eggs. Striped bass eggs were shown to hatch successfully at salinities up to 15 ppt, whereas levels

between 15 and 21 ppt resulted in larvae that did not survive (Winger and Lasier 1994). The mixing of freshwater flowing downstream with saltier ocean waters in bays and estuaries will likely result in salinities less than 20 ppt. Therefore, salinity levels in areas below dams that are tidally influenced will likely not affect hatching, even during high tides. This study also indicated yolk-sac larvae would be tolerant to tidally influenced waters, because they were able to survive all levels of salinity tested (0-30 ppt). This is atypical of many anadromous species, which tend to develop salt tolerence at metamorphosis (Zydlewski and McCormick 1997; Zydlewski and McCormick 2001; Ewing et al. 2001). This difference may be due to different outmigration patterns between smelt and other anadromous fish species. For example, shad are known to make very long spawning migrations upriver, up to 192 km from the river's mouth (Layzer 1974). Smelt, however, do not take these extensive migrations; they typically only go a few hundred meters above the tidewater (Bigelow and Schroeder 1953). Due to the proximity of smelt eggs to the more saline waters, larvae must be better adapted for osmoregulation at an earlier stage. Overall, salinity does not seem to have a significant impact on smelt early life stages; therefore, restoration efforts need not exclude brackish areas.

Nitrates and phosphates have different effects on varying fish species, but no visible affect on survival of smelt eggs and larvae was seen up to levels of 29.18 mg l-1 nitrate and 4.17 mg l-1 phosphate in this study. Nitrate levels of 5-10 mg l-1 are mildly toxic to early life stages of rainbow and steelhead trout;

however, nitrates did not affect egg hatch in Chinook or coho salmon, (Kincheloe et al. 1979). Phosphate levels up to 0.12 mg l-1 allowed common carp to hatch normally, but levels above 0.12 mg l-1 reduced hatch and abnormalities in larvae were observed (Toor et al. 1983). Two species of flatfish (*Limanda yokohamae* and *Paralichtys olivaceus*) were found to only be sensitive to levels of nitrates and phosphates between 100 and 1,000 mg l-1 (Yasunaga 1976), though these values are much higher than levels that would be found naturally. The increased use of fertilizers and construction of sewage treatment facilities along waterways have caused a rising concern for early life stages of fish influenced by escalating nitrate and phosphate levels. Nitrate (as N) and phosphate (orthophosphate as P) levels in New Hampshire rivers have reached 0.317 mg l-1 and 0.7203 mg l-1 respectively (NHDES National Coastal Assessment Tidal Water Quality Monitoring Data). Although no direct effects were seen on smelt eggs or larvae, further research should be conducted to examine possible negative effects on adult females and their eggs through endocrine disruption. This warrants further investigation, because Guillette and Edwards (2005) suggested that steroidogenesis and steroid biotransformation can be affected by nitrate exposure.

Field Comparison of Squamscott and Winnicut Rivers

The plates from the Winnicut River had a higher silt coverage and a greater reduction in egg viability than those in the Squamscott River. Jackson

(1952) suggested that the silt brought in on the high tides may prevent smelt eggs from adhering sufficiently to the rocks or may smother eggs that have already settled. Spawning substrate is vital to the health of the spawning runs, and under low flow conditions settling of fine sediments and an increase of periphyton degrades this substrate (Massachusetts Department of Marine Fisheries 2000). The silt may also cause a suffocation of the eggs. In order to respire in areas of lower flow and reduced oxygen, a natural convection current occurs around the egg, providing a continuous supply of oxygenated water. The carbon dioxide-rich water in the boundary layer around the egg sinks and is replaced by the less dense oxygen-rich water (O'Brien et al. 1978). When silt settles out onto the eggs, the convection around the egg may be hindered or completely stopped depending on the amount and size of the silt. Therefore, restoration efforts should include reduction of siltation on spawning grounds by opening non-tidally influenced areas to spawning or increasing flow on spawning grounds below dams.

The Squamscott River had a significantly lower change in viability than the Winnicut River, despite a greater amount of fungus. Rothschild (1961) found that the fungus itself did not reduce survival of the eggs; however, the mycelia would entrap the hatching larvae and prevent them from escaping the egg mass. So even though viability was not reduced as much as in the Winnicut River, successful hatching may be impaired. In areas with fewer regions of acceptable substrate or areas below impassable dams, overcrowding of eggs may occur.

Higher fungal growth in these areas may cause declines in the proportion of successful egg hatch (McKenzie 1947). Fungal growth and colonization can be minimized with proper water circulation and aeration (Akeilaszek et al. 1985). Again, increasing flow and opening more areas for spawning would likely increase recruitment by reducing mortality caused by overcrowding and fungal growth.

Of all the abiotic factors examined in this study, siltation appeared to be the most detrimental to early life stages of rainbow smelt. Dissolved oxygen, pH, and salinity did not have a negative effect until extreme levels were reached, which are unlikely to occur naturally on an annual basis. In addition to the siltation, fungal growth may also play a role in decreased successful hatch and thus recruitment. These factors may be intensified in areas below dams due to altered flow rates, overcrowding of eggs, lack of vegetation, and increased amounts of fine particles. Restoration could include altering discharge from the dams in order to increase flow during the spawning season, varying the riverbed by adding gravel to reduce fine particles, and dam removal to allow access to more spawning grounds in order to decrease fungal growth caused by overcrowding.

CONCLUSIONS

Of all the abiotic factors examined in this study, depressed dissolved oxygen had the most notable affect on shad eggs, while both dissolved oxygen and salinity influenced shad larvae. This study suggests emigrating American shad (*Alosa sapidissima*) released in the upper reaches of the Exeter River will have a decreased probability of reaching adulthood due to dams and their associated impoundments. Shad eggs and larvae will likely experience reduced survival when faced with a lack of overflow or poor water quality behind impassible dams. In addition, upstream migration of adults may be restricted by inadequate fishways; therefore, spawning may occur too close to the river mouth and progeny may be exposed to saline waters prematurely. Future endeavors should include improvements to downstream fish passage as well as to water quality conditions in the Exeter River to increase American shad growth and subsequent survival. Also, adult movement through the fishways should be monitored in order to ensure that fish can travel far enough upstream to spawn. Future studies conducted on outmigration of juveniles should be performed in years with a more normal discharge and with a more intense sampling regime. This study combined with the future recommendations would reduce costs and time and result in more successful restoration attempts and thus an increase in shad stocks.

The abiotic factors examined in the laboratory studies (dissolved oxygen, pH, salinity, nitrates, and phosphates) did not have an effect on survival of early life stages of rainbow smelt (*Osmerus mordax*) until extreme levels were attained, if at all. The field studies, however, indicated siltation and fungal growths may be detrimental to early life stages of rainbow smelt resulting in reduced recruitment of the species. The siltation and fungal problems may be intensified in areas below dams due to lower flow rates, overcrowding of eggs, lack of vegetation, and increased amounts of fine particles. Further studies investigating conditions influencing spawning success and recruitment need to be conducted in order to effectively restore smelt stocks. These studies should focus on the siltation and fungal growth occurring on spawning grounds. Not only should factors such as deposition in varying water flows be investigated, but also varying substrates should be tested. In addition, spawning site selectivity and egg survival were shown to be positively correlated to water velocity (Clayton 1976; Massachusetts Department of Marine Fisheries 2000; Sutter 1980); therefore, water velocity should be examined at the mouths of the rivers during spawning migrations. The reduced flow caused by the construction of the dams could cause adults to avoid these rivers to spawn.

Despite the fact that environmental parameters affect different species of anadromous fish in varying ways, the knowledge of harmful levels for any species will aid restoration of anadromous fish as a whole. For example, the most influential conditions, dissolved oxygen and salinity, on shad early life

stages were not as detrimental to smelt early life stages. This may be due to the manner in which incubation occurs. Shad eggs drift with the current until being lodged in gravel or rubble often traveling 1.6 to 6.4 km from where they were spawned (Marcy 1976). Because the eggs drift and settle, they would be surrounded by adequate water flow and therefore naturally higher oxygen saturations. Smelt eggs, on the other hand, are pedicellate, attaching to the substrate with a stalk. Therefore, they must be able to tolerate dissolved oxygen saturations around whatever substrate to which they are adhered. In addition to the varying effects of dissolved oxygen on these anadromous species, salinity tolerances were also found to differ between shad and smelt. Shad larvae were much more vulnerable to high salinities than the smelt larvae. Shad are known to make very long migrations upriver; Layzer (1974) observed spawning 192 km above the river's mouth. Smelt, however, do not take these extensive migrations; they typically only go a few hundred meters above the tidewater (Bigelow and Schroeder 1953). Because smelt spawn so close to the higher salinity estuaries, it would be advantageous for the larvae to have a higher salinity tolerance than the shad. Regardless of life history, the improvements to dams must occur before restoration attempts will be successful. By understanding what affects the dams are having, improvements such as increasing flow and allowing fish to access more potential spawning grounds can be made to provide all anadromous fish with a more natural spawning environment.

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APPENDICES.

ANIMAL CARE AND USE APPROVAL DOCUMENTATION

APPENDIX A. SHAD

UNIVERSITY OF NEW HAMPSHIRE

Office of Sponsored Research
Service Building
51 College Road
Durham, New Hampshire 03824-3585
(603) 862-3564 FAX

LAST NAME	Berlinsky	FIRST NAME	David
DEPT	Zoology, 171 Spaulding	APP'L DATE	3/30/2004
OFF-CAMPUS ADDRESS (if applicable)	Zoology, 171 Spaulding	IACUC #	040305
		REVIEW LEVEL	B
		TODAY'S DATE	4/9/2004
PROJECT TITLE	The effects of Passage Impediments and Environmental Conditions on Out-Migrating Juvenile American Shad		

All cage, pen or other animal identification records must include your IACUC Protocol # as listed above.

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the protocol submitted for this study under Category B on Page 4 of the "Application for Review of Animal Use in Research or Instruction" - the study involves either no pain or potentially involves momentary, slight pain, discomfort or stress.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please note: Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. ***Participation is mandatory*** for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Van Gould at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Roger Wells, D.V.M.
Vice Chair

cc: File

UNIVERSITY OF NEW HAMPSHIRE

Office of Sponsored Research
Service Building
51 College Road
Durham, New Hampshire 03824-3585
(603) 862-3564 FAX

LAST NAME	Berlinsky	**FIRST NAME**	David
DEPT	Zoology (UBZOOL)	**NEXT REVIEW DATE**	1/29/2005
OFF-CAMPUS ADDRESS (if applicable)	Spaulding Hall	**IACUC #**	030101
		REVIEW LEVEL	B
		DATE OF NOTICE	12/4/2003

PROJECT TITLE Development of practical culture methods for rainbow smelt

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved your request for a time extension for this protocol. Approval is granted until the "Next Review Date" indicated above. You will be asked to submit a project report with regard to the involvement of animals before that date. If your project is still active, you may apply for extension of IACUC approval through this office.

The appropriate use and care of animals in your project is an ongoing process for which you hold primary responsibility. Changes in your protocol must be submitted to the IACUC for review and approval prior to their implementation. If you have questions or concerns about your project or this approval, please feel free to contact the Regulatory Compliance Office at 862-2003 or 862-3536.

Please note: Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. *Participation is mandatory* for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

Please refer to the IACUC # above in all correspondence related to this project. The IACUC wishes you success with your research.

For the IACUC.

Jessica Bolker

Jessica A. Bolker, Ph.D.
Chair

cc: File ORIG APP'L 1/29/2003